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TABLE OF CONTENTS

Arthur Henry Reginald Buller.
W. F. Hanna, C. W. Lowe, and E. C. Stakman 577

The effect of rugose mosaic on the yield of potatoes ..... J. G. Bald 585

Virus C from an old Australian variety of potato.
J. G. Bald and D. O. Norris 591

Tobacco anthracnose, a plant bed and field disease.
E. A. Walker and Elizabeth Wise McInroy 598

Curly-top and California-aster-yellows diseases of flax.
Henry H. P. Severin and Byron R. Houston 602

Transmission of peach wart to sweet cherry.
S. M. Zeller and J. A. Milbrath 607

Anatomy of buckskin-diseased peach and cherry. Henry Schneider 610

A root rot of guayule caused by Pythium ultimum.
W. A. Campbell and Bailey Sleeth 636

A simple method of inoculating barley with loose smut.
J. M. Poehlman 640

Smut control in sorghum and effect of dust fungicides and storage on emergence ..... R. W. Leukel and J. E. Livingston 645

Report of the 1945 annual meeting of the New England Division of the American Phytopathological Society 654

ARTHUR HENRY REGINALD BULLER
1874–1944

W. F. Hanna, C. W. Lowe, and E. C. Stakman

Professor Arthur Henry Reginald Buller was born in Birmingham, England, August 19, 1874, and died in Winnipeg, Manitoba, July 3, 1944, of a brain tumor. During a long and uninterrupted career as an investigator and teacher his contribution to science, particularly in his chosen field of mycology, was prodigious. And yet, despite his extraordinary ability and industry, he left much unfinished work, a possibility about which he had often expressed concern.

After obtaining the B.Sc. degree from the University of London in 1896, Buller was awarded an 1851 Exhibition Scholarship which enabled him to study under Pfeffer in Leipzig, where he was granted the Ph.D. degree in 1899. He also studied with Hartig in Munich and later spent some time at the Marine Biological Station at Naples. Buller often paid tribute to the stimulating influence of both Pfeffer and Hartig. He reminisced especially about his field trips with Hartig and cherished recollections of his close association with this pioneer in the field of forest pathology.

From 1901 to 1904 Buller was lecturer in botany at the University of Birmingham and in the latter year went to the University of Manitoba at Winnipeg as professor of Botany, where he remained until his voluntary retirement as professor emeritus in 1936. When asked how he happened to go to Winnipeg, Buller said that he had seen in some literature about Canada a description of "Winnipeg, The Mushroom City" and had immediately realized that it would suit his tastes and interests so nicely that he decided to cast his lot with the thriving young metropolis of the Canadian prairies. And there he taught his students and immersed himself in his researches on fungi.

Although he crossed the Atlantic 65 times, in order to spend his summers in England, Buller formed a strong attachment for Canada and worked hard for the development of his University and the Dominion Laboratory of Plant Pathology, established originally in 1923 as the Dominion Rust Research Laboratory. The summer of 1939 found Buller in England, but early in September of that year he came to New York to attend the meetings of the International Microbiological Congress. At the close of the Congress he decided, because of the difficulties of ocean travel, to return to Winnipeg where he remained until his death except for occasional absences for lectures and special courses at various American Universities. Had it not been for the war, Buller probably would have taken up permanent residence in England and there continued with his writing and research.

As a teacher Buller was a perfectionist. He prepared lectures and laboratory materials with meticulous care and attempted to make every lecture a finished performance. Likewise, he considered it an obligation to

ARTHUR HENRY REGINALD BULLER
1874–1944

make each laboratory period a really valuable experience for the students. He was as exacting of himself as of his students. Slovenliness of any kind, mental or physical, was abhorrent to him. Orderliness and preciseness were among his cardinal requirements. To Buller the lecture room and laboratory were sacred to science, and he tolerated no intellectual inattention or personal misbehavior. Many students have testified to the disciplinary values of his courses. And yet Buller himself was so interesting, so genuinely enthusiastic in his teaching, and had to so high a degree the faculty of making botany interesting and significant that serious students evaluated his teaching not only as an intellectual discipline but also as a stimulus to the expansion of mental horizons. This is the consensus of a random sample of former undergraduate students after a lapse of time had given them perspective on educational values.

Buller's attitude towards post-graduate study and research was profoundly affected by the environment in which he moved during the early years of his scientific career. As a young science student he became absorbed in Darwin's great work on the Origin of Species and was deeply influenced by Huxley's brilliant advocacy of the theory of evolution and his defense of the scientific method. In this stimulating environment science was revealed to Buller as something more than a métier; it was a never-ending search for truth, a thrilling adventure of mind and spirit in new fields of knowledge, with fresh vistas ever stretching beyond the horizon; it was for him a way of life. At the time Buller went to Germany to study with Pfeffer and Hartig, the wholesale application of science to industry and agriculture, necessitating as it has done the training of great numbers of specialized scientific workers, had scarcely begun. The number of graduate courses available to research students was strictly limited, and the relationship between professor and student was much more intimate than is possible in the scientific laboratory of today. The high degree of specialization which characterizes present-day scientific study was unknown in the late nineties and indeed, in this regard, it may be recalled that Buller's early scientific training and interests covered the fields of zoology and geology, as well as that of botany. With such a background, then, it was but natural that Buller viewed with a mixture of regret and understanding the growing tendency towards specialization in the various fields of applied science. In later life when his work was widely known and his position in the scientific world was firmly established, he might have built up a large graduate school, but he preferred instead to give his attention to a relatively small number of students whose work he could closely supervise, while at the same time continuing with his own writing and research.

Buller's relationship with his graduate students was always warm and intimate. He was a friend and counselor as well as a stimulating teacher. His enthusiasm was contagious, his energy untiring, his way of life both unconventional and unpredictable. A confirmed bachelor, he was in every sense of the word "unattached." If some interesting experiment was to be

attended to or if a fruitful new idea came to his mind he might appear in the laboratory at five or six o'clock in the morning and work furiously throughout the day or even until after midnight, stopping only for a lunch of tea and biscuits at his desk and perhaps forgetting entirely about dinner. But when the task was accomplished, Buller had the happy faculty of being able to relax completely and he might then indulge in his favorite recreations—a game of billiards, a cigar, a turn at the piano, a half hour with Shakespeare, Keats or Shelley, a good novel, an occasional movie, or a stroll among the trees and flowers. While always tolerant of others he perhaps never fully understood the predicament of more orthodox individuals whose way of life seemed to conform to a fixed pattern and whose comings and goings were regulated by habit rather than by the task at hand.

Buller was essentially a student of Nature and of living things. He was in no sense a systematist, although his knowledge of plant and animal species was remarkably complete. His chief interest lay in elucidating the living processes of organisms, their methods of reproduction and dissemination, and their relationships to environment. To him laboratory studies were merely a means to this end and he never failed to relate his laboratory findings to his observations in the field. Not a few of his most important laboratory discoveries were suggested by field observations. Trained in a period when botanists relied mainly on the microscope, the pencil, and the free-hand section, he regarded with some suspicion the conclusions which were occasionally drawn from the study of fixed and stained microtome sections. He was adept at cutting free-hand sections of living material and from them he made numerous and accurate sketches later to be converted into the beautiful line drawings with which his Researches and scientific papers are so copiously illustrated. He worked rapidly, his powers of observation were amazing, and he never lost sight of the possible significance of the most trivial observation.

Although endowed with a keen imagination, the imagination of an artist, Buller never allowed his imaginative faculties to dominate his reason. His fund of ideas was seemingly inexhaustible and his students would sometimes almost despair when, in the course of a brief conversation, he might suggest a multitude of interesting experiments which nevertheless would require months to carry out. He speculated freely, but he was always eager to submit his hypotheses to the acid test of experimentation.

In the course of his scientific career Buller developed a clear, easy, and forceful style of writing. He was ever willing to assist his students in the preparation and revision of their papers and, while avoiding undue interference with the individual's methods of expression, he nevertheless insisted upon the observance of certain fundamental rules. Accuracy and clarity were for him the prime requisites of good scientific writing. He made a practice of reading his own manuscripts aloud to his students and invited their criticism and comment. To the novice who sought in vain a precise word or a nicely turned phrase he gave the encouraging assurance that he

himself in his student days had experienced no less a difficulty. And yet to all who knew him it was evident that the ease with which he expressed his thoughts in both verse and prose could not have been acquired by practice alone, but stemmed from a fine natural feeling for the choice and meaning of words.

Perhaps the most vivid impressions which graduate students retain of Buller are his enthusiastic devotion to science, his love of Nature, his kindliness, and his generosity to those who needed a helping hand. Above all he was an English gentleman in the finest sense of the word. Although he came to have a deep attachment for Canada, where he spent nearly forty years of his life, he always remained at heart an Englishman and the last years of his life were saddened by the destruction wrought by war in his native land which ever was for him:

> This precious stone set in a silver sea—
> This blessed plot, this earth, this realm, this England.

Research was a passion with Buller: Few men could have had for it such single and deep devotion. He worked to the limit of his strength and sometimes beyond. Buller reduced the mechanics of living to its simplest terms in order that he might be completely free to devote himself to his science, and his attainments are evidence that he must have worked with virtually maximum efficiency most of the time. He often said that he had a poor memory; but his rich store of information belied his assertion. Native curiosity, acuteness of observation, and everlasting persistence led him to discover many obscure and apparently insignificant facts. But nothing in Nature was insignificant to Buller. Through his powers of synthesis he wove many of these apparently insignificant facts into highly significant principles. He was sometimes twitted with being a teleologist; if his viewpoint was teleological, it certainly paid dividends, both in his researches and in his ability to dramatize the life of the fungi. He was, of course, not a teleologist in the invidious connotation of the word: rather he looked always for relationships between phenomena and for the utility of structures and processes. He reasoned that there must be underlying causes for the phenomena which he observed and he was not satisfied until he found them.

Researches on Fungi, six volumes filled with a multitude of interesting and important facts and ideas about fungus behavior, presented in an easy, lucid style, constitute a monument to Buller's genius. He made fascinating stories out of spore production, spore liberation, and spore dissemination. He studied sex, so-called social organization, hyphal fusions, protoplasmic streaming, and nuclear associations. His pen pictures were extraordinary in a double sense; he was an artist, verbally and graphically. But Researches on Fungi are not only interesting; for they contain much that is valuable in a practical way. His studies on spore formation, liberation, and dispersal pointed the way, even though indirectly, to a better comprehension of some of the factors affecting the development of plant-disease epidemics. And yet, his books are a treasure of information quite apart

from any values other than those that satisfy an inquiring mind or cause it to wonder at the marvels of structure and function of some of the most fascinatingly mysterious of all living organisms. For Buller himself often marvelled at the wonderfully nice adaptations and adjustments that enable mushrooms to crowd prodigious numbers of spores on their hymenial surface and then ensure their liberation. Buller was a scientist; therefore he studied as closely as possible the mechanisms involved. But he was a philosopher too; and he wondered and speculated about the nature of the genes that directed the building of the structures and the functioning of the mechanisms. And this intense desire not only to know what happened but also why it happened impelled him to delve more deeply than most into the intimate life of the fungi. With infinite pains and infinite patience he accumulated a vast store of knowledge about things that had been taken for granted. For Buller took nothing for granted.

Professor Buller was in great demand as a lecturer: he gave many individual lectures and held a number of special lectureships in the United States. He was Norman Wait Harris Foundation lecturer at Northwestern University in 1927, summer lecturer at Louisiana University in 1941, Hitchcock professor at California in 1942, and in the same year Schiff Foundation lecturer at Cornell.

Scientific affiliations included membership in the American Phytopathological Society, Botanical Society of America (president in 1928), British Association, British Mycological Society (president in 1913), Canadian Phytopathological Society (president in 1920), and in the Society for Psychical Research. He was associate member of the Société Royale de Botanique de Belgique, corresponding member of Société Botanique Neerlandaise, honorary member of the Indian Botanical Society, and Fellow of the Royal Society of Canada (president in 1928). In 1929 he was elected Fellow of the Royal Society of London.

The honorary degree of Doctor of Laws was conferred upon him by the University of Manitoba in 1924, by the University of Saskatchewan in 1928, and by the University of Calcutta in 1938. Pennsylvania University granted him the degree of Doctor of Science in 1933. He was recipient of the Flavelle medal from the Royal Society of Canada in 1929, the medal of the Natural History Society of Manitoba in 1936, and the Royal Medal of the Royal Society in 1937.

Buller's interests were many and varied. He loved to stroll in the woods and fields, sometimes to make professional observations, but quite as often to commune peacefully with Nature. He was fond of birds and noted their habits with keen interest. His days were usually spent in teaching, writing, and research, but in his leisure moments he was a discriminating reader of good prose and verse. Endowed with the gift of expressing his thoughts in verse as well as in prose, he left behind a sizeable collection of poems, rhymes, and limericks, many of which have real merit. Always a lover of books, he gathered together a fine selection of botanical works, including

many rare and interesting volumes on the fungi. His recreations included billiards, which he played with skill and enthusiasm, playing the piano for his own amusement or to entertain his friends, and occasional tricks of legerdemain. Withal, he had a good sense of humor and a kindly nature which, with his little quirks of character, made him always an interesting and entertaining companion.

In his chosen field of science, Buller attained preeminence. The last of his vivid lectures has been given, but his published works remain as a remarkable record of achievement of an extraordinary scientist and as an inspiration for present and future students of the fungi.

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THE EFFECT OF RUGOSE MOSAIC ON THE YIELD OF POTATOES

J. G. Bald

(Accepted for publication December 27, 1944)

DESCRIPTION OF THE EXPERIMENTS

In two field experiments at Canberra some potato plants of the variety known in Australia as Early Carman (probably Green Mountain), grown from tubers thought to be healthy, were found to be infected with rugose mosaic. For the original purpose of these experiments, periodical measurements of leaf area were taken on each plant (1, 3), and also a record of the final yield. Data of this sort provided information about the growth of the foliage, and about the relation between leaf area and yield of diseased and normal plants.

The plants were grown in the same poor sandy loam in different seasons, one lot during the early portion of the season 1941–42, and the other in the later portion of 1942–43. The original experiments have been described elsewhere (1, 3, 5).

In both instances the conditions of growth were far from optimal. The weather was hot and dry, and the light intensity high. The plants had to be watered to maintain growth. In the first experiment, during the later stages of development the plants received barely enough water to allow them to finish their growth. In the second experiment, from the time of flowering onwards, the foliage of the plants was damaged by larvae of the potato moth. However, there is no reason to suspect that these conditions seriously affected the comparison between the diseased and normal plants. The environment in which the plants grew may be summarized as one in which the efficiency of photosynthesis was likely to be high and the absorption of water and nutrients from the soil was likely to be the limiting factor for growth, even of diseased plants.

In the 1941–42 experiment there were 10 plants infected with rugose mosaic, and 20 plants, in the same small plots, that were normal throughout the season. These fell, according to their position in the experimental area, into equal groups of 5 diseased plants and 10 normal. The "normal" plants contained virus X, and the diseased plants a mixture of viruses X and Y. The comparison was thus not between diseased and completely healthy material, but between plants carrying latent and obvious infection.

In the experiment of 1942–43, six normal Early Carman plants and two infected with rugose mosaic were studied in detail. The development of the individual leaves on the main and axillary shoots was followed until the maximum leaf areas were attained, and estimates of the total leaf area were obtained throughout the whole period of growth from emergence to senescence (3, 5). Owing to the conditions of temperature and daylength late in the season when the plants were grown, the total period of development was only about two-thirds as long as in 1941–42.

RESULTS

In figure 1 is shown for the 1941–42 experiment the leaf area of the two groups of diseased and healthy plants from October 24 to December 20. They reflect differences in soil moisture in the portions of the experimental area where they grew. The growth of group A was checked soon after the

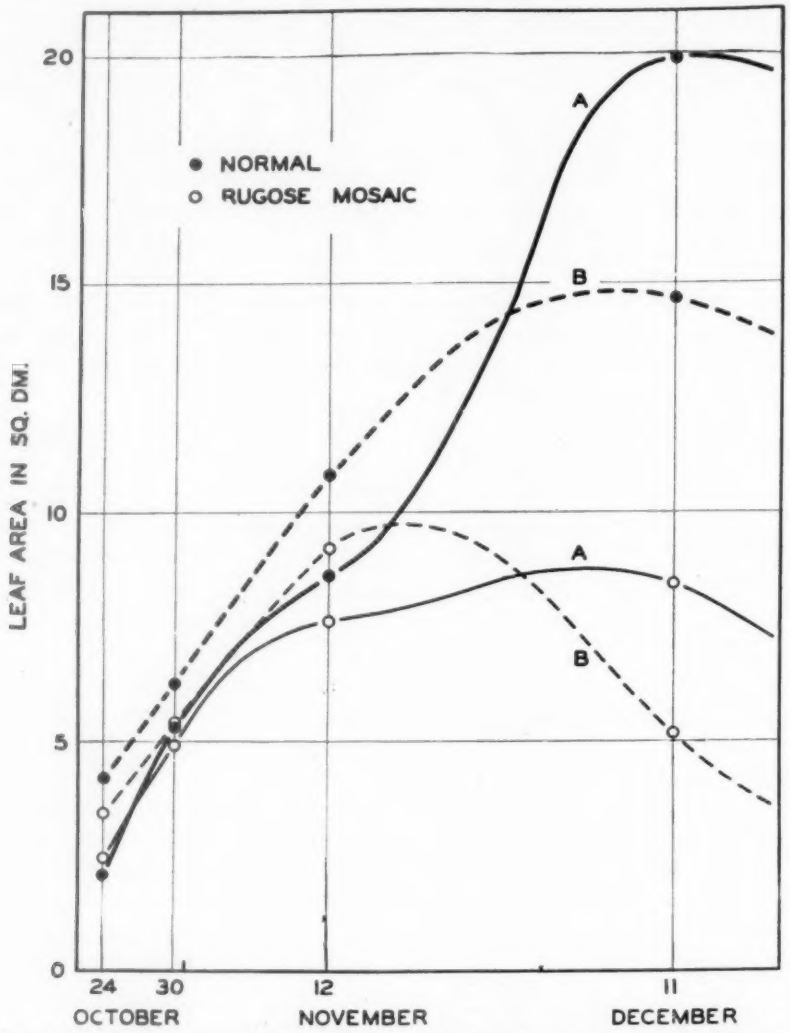

FIG. 1. Mean leaf area of potato plants infected with rugose mosaic (viruses X and Y) and "normal" plants (containing virus X). There were two groups of plants, A and B, from different parts of the experimental field. In each group were five diseased plants and ten healthy. From 1941–42 experiment.

inception of flowering, but later, after being irrigated, the normal plants increased rapidly in size, and reached a greater maximum leaf area than the normal plants in group B. The diseased plants of group A also made fresh growth, but at the same time they lost some of their lower leaves. The leaves

shrivelled in the manner usual, during the later stages of development, with mosaic-infected plants of this variety. The effect of the loss of leaves is clearer in the growth curve for diseased plants in group B, the leaf area of which reached a clear maximum and then declined.

During the earliest stages of growth, these diseased plants revealed little evidence of infection and increased in size at almost the same rate as the normal plants. Later, as symptoms became evident, the growth rates declined in comparison with those of the normal plants. By November 12, the mean leaf area of the diseased plants was 14 per cent below that of the normal plants, and the difference was significant. In the combined groups, A and B, the mean of the maximum leaf areas reached by the diseased plants was little more than half the maximum for the normal plants.

The total yields in table 1 show differences between diseased and normal plants of the same order as for the mean maximum leaf area. The ratio

TABLE 1.—*Yield of Early Carman plants infected with rugose mosaic (viruses X and Y) and normal plants (infected with virus X); 1941–42 experiment*

Groups and state of plants	No. plants	Yield in gm. per plant						Yield in gm. per sq. dm. of leaf area
		Tubers below 2 oz.		Tubers above 2 oz.		Total		
		No.	Wt.	No.	Wt.	No.	Wt.	
A. Diseased	5	2.2	64	1.6	115	3.8	179	8.8
Normal	10	1.9	72	3.1	265	5.0	337	8.7
B. Diseased	5	3.0	104	1.4	97	4.4	201	10.7
Normal	10	2.7	96	3.0	299	5.7	395	11.2

between maximum leaf areas and yield in the two blocks varied considerably, but the variation arose mainly from the different forms of the foliar growth curves. A better estimate than the maximum leaf area of the efficiency of the leaves in the formation of tubers is given by the integration of the values for leaf area over the period from the inception of flowering (which may also be taken as the inception of tuberation) until the last estimate of leaf area, *i.e.*, for approximately six weeks from November 10. The simplest method of integration is to find the mean leaf area over this period. Values for the yield per sq. decimeter of mean leaf area are in table 1. Equal areas of leaf on diseased and normal plants produced very nearly the same weight of tubers.

Infection with rugose mosaic caused a greater fall in the weight of total yield than in the number of tubers. The yield of tubers below marketable size was about equal for diseased and normal plants, and therefore the loss in total yield due to virus Y was less than the loss in marketable yield. The loss in marketable yield was about 63 per cent.

Diseased plants in the second (1942–43) experiment developed symptoms immediately on emergence, and were severely dwarfed. This is characteristic of plants from infected tubers that have been sprouted, greened, and

held for a considerable time at fairly warm temperatures. In the 1942–43 experiment, the mean leaf area of the two infected plants was little more than one-tenth the mean leaf area of the six normal plants.

As the growing period in this experiment was only about two-thirds that of the plants in the earlier experiment, the leaf area during a period of 30 days instead of six weeks after the beginning of flowering was integrated by taking the mean. The yields were weighed to the nearest five grams. The results for the individual plants, six normal and two diseased, are in table 2.

As in the previous experiment, there was no demonstrable difference between diseased and healthy plants in the yield per sq. decimeter of leaf area. Amongst the plants from numbers 7 to 14, which included the two diseased plants, the variation in the values was negligible. The healthy

TABLE 2.—*Mean leaf area over a period of 30 days from the initiation of flowering, and total yield of six normal Early Carman plants, and two infected with rugose mosaic; 1942–43 experiment*

State of plants	Position in row	Mean leaf area (sq. dm.)	Yield	
			(gm.)	(gm./sq. dm.)
Normal	3	10.6	115	10.9
	6	28.8	330	11.1
	8	17.9	290	16.2
	13	18.6	205	16.4
	14	29.0	470	16.2
	15	19.2	270	14.1
Diseased	7	1.76	30	17.0
	10	4.65	70	15.1

plants 3 and 6, and, to a lesser extent, 15 gave lower values, probably because of a deficiency of moisture in the soil where they grew.

The results of the leaf area measurements agree with those of Stone (7) made on similar diseased material, and need not be given in detail. As the leaf area measurements were carried beyond the stage of development studied by Stone, several points may be added to her description of the foliar growth. Even on the diseased plants of the 1941–42 experiment, on which symptoms were slow to appear, there was not the usual number of axillary shoots. Those that did form were small. The reduced axillary growth and the premature shrivelling of the lower leaves were the main causes of the reduced leaf area of diseased plants. In the 1942–43 experiment, the diseased plants produced practically no axillary shoots, and the leaves on the main axes were very small. The loss of the basal leaves was hastened by the attack of potato moth larvae. However, the normal plants were attacked in a similar manner (5).

DISCUSSION

Among the potato plants diseased with rugose mosaic there was a number of indications of restriction in the plants' supplies of available protein.

Firstly, there was the reduction in leaf area associated with infection. In healthy plants of related genera, leaf area is a fair measure of protein content in the pre-senescent stages of growth (6). Presumably the manufacture of virus protein replaced not only the manufacture of reserve protein (9), but also the manufacture of some of the cytoplasmic protein necessary for the formation of new centers of metabolism in the leaf tissues. The reduction of axillary growth on diseased plants in the first experiment and its almost complete absence in the second suggest that the available protein in diseased plants was insufficient to support, in addition to tuber formation, the development of tissues from secondary growing points. The premature shrivelling of the lower leaves suggests that even the cytoplasmic protein they contained was hydrolyzed to maintain growth elsewhere, or for the manufacture of additional virus protein (8).

On the other hand, the functional leaf area measured during the pre-senescent tuber-forming stage of development appeared as efficient in the formation of tubers on plants diseased with rugose mosaic as on "normal" plants containing only virus X. Apparently there was no inhibition of translocation in the diseased plants, and the efficiency of the centers of metabolism actually formed in the leaves of diseased plants, in so far as it was reflected in the formation of tubers, was not further reduced by the imposition of virus Y on the original infection with virus X.

These results, on the surface, contradict those obtained by Stone (7), who found that mosaic-infected plants produced a smaller weight of tubers per unit of leaf area than the normal control; but there are points of difference between the American and Australian experiments that make a direct comparison of results difficult. The yields obtained by Stone were not mature yields, and it is probable that the course of tuber development is different in normal and mosaic plants. Also, leaf areas were calculated only for the earlier phases of development, and the means were partly derived from daily values obtained before the stage at which tuberation begins. If, in the experiments described here, the final yield is divided by the mean leaf area over a period equivalent to that used in Stone's calculations, the yield per sq. decimeter of leaf area is considerably greater for normal than for diseased plants. For example, in the 1941–42 experiment, the mean figures are 43.1 and 26.8 gm. per sq. decimeter.

There thus appear to be two levels in the reduction of yield due to the mosaic diseases of the potato. At the first level, represented by infection with average strain mixtures of virus X, the virus appears to multiply in the host at the expense of reserve proteins only, as the foliar portions of the plant grow to the normal size (2, 4). Loss of yield is presumably due to the inability of the plant to hydrolyze the virus protein for translocation to the tubers. At the second level, represented by plants infected with rugose mosaic, the competition exerted by the metabolism of virus protein against the normal protein metabolism is sufficient not only to replace reserve protein by virus protein, but also to replace protein needed for the establish-

ment of new centers of metabolism. This results in a smaller leaf area on diseased plants, and an enhanced reduction of yield.

SUMMARY

In two experiments, leaf areas were measured on potato plants infected with rugose mosaic, due to viruses X and Y, and on "normal" plants containing virus X. Final yields were also measured. The reduction in yield due to virus Y was proportional to the reduction in leaf area measured during a period after the initiation of tuber formation. The yield of tubers over 2 oz. in weight was reduced by infection with virus Y more than the total yield, because the reduction in numbers of tubers was proportionately less than the reduction in leaf area.

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VIRUS C FROM AN OLD AUSTRALIAN VARIETY OF POTATO

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INTRODUCTION

In November, 1938, studies were begun on a virus that had been isolated several times from an old Australian potato variety, Brown's River (1). Its properties and reactions corresponded with those given in the meager descriptions of virus C then available (2, 6). As the Brown's River virus was studied in detail, it was found also to be closely similar to virus Y. Apart from its reactions on the standard varieties of potato, the only property found to differentiate it from virus Y was the inability of the aphid, *Myzus persicae* Sulz., to transmit it to healthy plants of susceptible varieties, under conditions in which virus Y was readily transmissible.

Even this distinction was not complete, as transmission of the Brown's River virus by *Myzus persicae* was recorded in two instances. On the other hand, although previous infection with virus Y usually prevented potato plants from reacting to the Brown's River virus with characteristic symptoms, there were instances in which we could not be sure that infection with virus Y had given complete protection. The evidence on the identity of the Brown's River virus was thus somewhat confused. Further experiments on its transmissibility and its relationship to virus Y were planned, but could not be made at the time. Since then, the published results of other workers (3, 5) and some more recent work at Canberra have removed doubts that the Brown's River virus is virus C, and that virus C must be considered a strain of virus Y.

SOURCE OF THE VIRUS

The Brown's River potato[1] is a late variety with reddish purple tubers and a vigorous habit of growth. The two commercial stocks examined were almost entirely infected with virus C or virus Y. Infection of Brown's River with virus C is often not detectable in the field, but in the greenhouse it causes a slight veinal mottle and rugosity of the leaves. Sometimes in the greenhouse the mottle is masked, and the only symptom is a slight rugosity, that can be distinguished by comparison with healthy plants. This comparison has been made possible by the isolation of virus-free stocks of Brown's River from single plant selections made by Dr. R. A. Scott of the Tasmanian Department of Agriculture.

SYMPTOMS ON POTATO

Brown's River has field resistance or immunity from virus X, and uncontaminated samples of virus C have readily been obtained from it by

[1] Other names applied to this variety were Brown's River Black, Circular Head, and Redskin. It was the principal maincrop variety in Australia during the nineteenth century.

inoculation to tobacco and other susceptible hosts. Although Bawden (2) originally stated that "virus C has not been transmitted to any plants by needle inoculation," Dykstra (7) found mechanical transmission to tobacco very easy by the methods he used, and Cockerham (4) states that mechanical

TABLE 1.—*Summary of the symptoms on 11 varieties of potato inoculated or grafted with material containing Brown's River virus*

Variety and means of transmission	Symptoms
President (graft)	Top necrosis.
" (inoculation)	Necrotic local lesions.
Up-to-Date (graft)	Top necrosis.
" (inoculation)	Necrotic local lesions (Fig. 1).
Arran Victory (graft and inoculation)	Very mild mosaic.
Epicure (graft)	Top necrosis, not always severe enough to kill the plant rapidly. From the tubers small plants were produced which became necrotic and died when they were a few inches high, or developed a dwarfing mosaic disease.
" (inoculation)	Necrotic local lesions, and occasionally systemic symptoms as above.
Katahdin (graft and inoculation)	A rather indefinite mottle in the current season, sometimes a certain amount of necrosis and shrivelling of the lower leaves; in the following season definite and characteristic crinkling and veinal mottle.
Seedling 41956 (graft)	Similar to Katahdin but more severe; no current season necrosis was observed.
Carman (graft)	Top necrosis.
" (inoculation)	Local necrosis, necrotic streaking of petioles and stem, top necrosis.
Brownell (graft)	Only one plant was grafted. No current season symptoms were observed, but in the following season a veinal mottle and rugosity developed, necrotic streaking on the undersides of the veins and shrivelling of the lower leaves.
Snowflake (graft)	Top necrosis; in the second season dwarfing, severe crinkle, necrosis and early death.
" (inoculation)	Local necrosis and retention patterns, caused by the loss of chlorophyll except in restricted areas of the leaf. Systemic symptoms were semi-necrotic, spreading slowly to the growing tip.
Bismarck (graft)	Severe mottle, rugosity, thin necrotic streaks on the undersides of the veins, shedding of the lower leaves until only a terminal tuft remained. In the second season, severe mottle, rugosity and some necrosis.
Delaware (Earliest of All) (graft)	Top necrosis beginning as necrotic spots or a necrotic network on the veins of the youngest leaves, streaking of the stems and collapse.

transmission to potatoes reacting without necrosis is obtainable. At Canberra, the Brown's River virus was transmitted both to certain varieties of potato and to other hosts by mechanical inoculation with a glass spatula and carborundum powder. For varieties of potato that did not react by necrosis, this method appeared reasonably effective, but not always so effective as similar inoculations with virus Y.

The varieties inoculated or grafted from the original Brown's River material were the four English test varieties, President, Up-to-Date, Arran Victory, and Epicure; the American varieties, Katahdin and U. S. seedling 41956; and the five varieties, which, apart from Up-to-Date, are most widely grown in Australia, Carman (possibly Green Mountain), Brownell (Adirondack), Snowflake, Bismarck, and Western Australian Delaware (probably Earliest of All). Their reactions are in table 1.

OTHER SOLANACEOUS HOSTS

Four common solanaceous hosts have been tested for their reaction to the Brown's River virus; tobacco, variety Hickory Pryor, *Nicotiana glutinosa, Datura stramonium,* and pepper. Of these, *N. glutinosa* gives the most

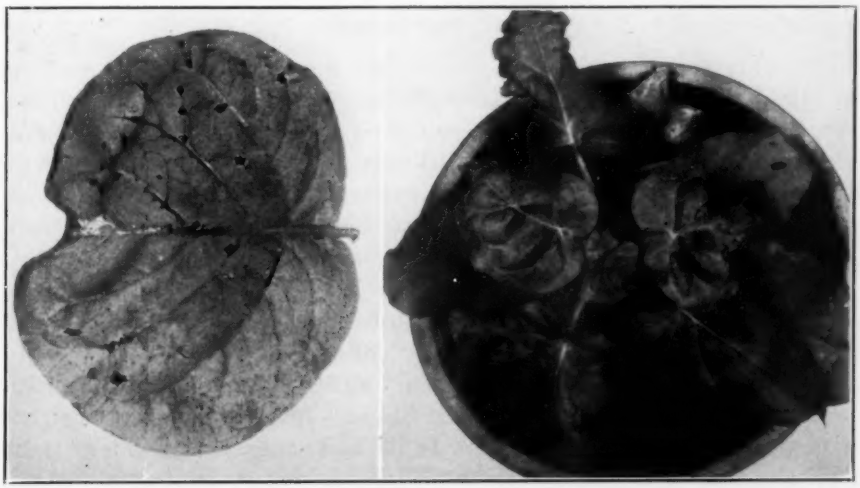

FIG. 1. *Left.* Necrotic lesions produced on inoculated leaves of Up-to-Date potato by the Brown's River virus. *Right.* Fully developed symptoms of the Brown's River virus on *Nicotiana glutinosa.*

characteristic reaction. Occasionally, 5 or 6 days after inoculation, indefinite yellow local lesions may be produced. Under normal conditions, after an incubation period of between 7 and 12 days, vein-clearing occurs, and within another week the areas between the veins balloon upwards, and a mottle develops consisting of vein-banding and a loss of color in the interveinal areas (Fig. 1). The plant grows on, but is stunted, and distortion of the younger leaves may be severe. Samples of virus Y inoculated in parallel series as a rule gave similar but less severe symptoms. The symptoms on tobacco are almost indistinguishable from those of virus Y. On *Datura* no infection has been produced by inoculation or grafting. On pepper infection occurs, but symptoms do not appear until about five weeks after inoculation. They consist of a mild but characteristic mottle; yellow-green areas extend from the main veins, vein-banding or green islands develop sparsely along the veins, and the mottle may be outlined by light bronze lines. The leaves may roll upwards, and become yellowish green.

The addition of virus X to the Brown's River virus in tobacco or *Nicotiana glutinosa* generally produces double symptoms like those characteristic of the virus X-virus Y mixture, *i.e.*, yellow vein-clearing, and more or less necrosis according to the strain of X used. In at least one instance, however, when the Brown's River virus was present in tobacco and virus X was added, the resultant symptoms were mild. In other instances, in which a mixture of the two viruses was inoculated to tobacco, the usual double-virus symptoms did not develop very clearly, and those due to virus X were predominant.

Six hosts outside the *Solanaceae* were inoculated with the Brown's River virus without producing any infection. These were cucumber, zinnia, and cowpea (which react to cucumber mosaic), pea, French bean, and broad bean.

INSECT TRANSMISSION

The only insect tested as a vector of the Brown's River virus has been the aphid, *Myzus persicae*, the most efficient vector of virus Y. Early experiments in which the aphids were fed on the shoots of sprouted tubers were negative, but so also were parallel tests with virus Y, although leaf roll was transmitted easily. Sprouting tubers are a poor source of virus Y, and probably of virus C. An indication that *M. persicae* might be a vector of the Brown's River virus was obtained in one instance when aphids feeding on an infected plant were transferred to President. On one leaf of one plant a necrotic lesion developed and spread down a main vein, eventually killing the leaf. Systemic infection did not occur.

In other experiments aphids were fed on diseased plants of potato varieties with a non-necrotic reaction to the Brown's River virus, and transferred to *Nicotiana glutinosa* and tobacco. In the first experiment of this type, one *Nicotiana glutinosa* of five inoculated became infected. Other transfers gave negative results.

In all, 16 transfers were made to varieties of potato reacting to the virus with necrosis, 27 to varieties with a non-necrotic reaction, 16 to *Nicotiana glutinosa*, 15 to tobacco, and 10 to pepper, a total of 84. About 20 aphids per plant were used for each one. Only in the two instances described did infection occur.

PHYSICAL PROPERTIES OF THE VIRUS

Dilution End-point

The results of four experiments are shown in table 2. The first two were performed at different times with extracts of recently infected plants of *Nicotiana glutinosa*, the last two with leaves from tobacco plants that had been infected for a number of weeks. The results show the usual diminution of concentration that occurs as infected plants age. The inoculum for the fourth experiment was extracted from the leaves by the chemical method Stanley used for the preliminary stages in the isolation of bushy stunt-virus (9). The reason for the failure of infection at the 1 in 5 dilution may have been the high concentration of phosphate in the inoculum.

These are much higher dilution end-points than are recorded by Kenneth Smith (8) for virus Y. Under our conditions, however, the dilution end-point of virus Y is of the same order as that of the Brown's River virus. This may partly be the result of more efficient methods of inoculation, but it is probably also a reflection of the high concentrations of virus that normally develop in host plants under the high light intensity and temperatures in the greenhouse at Canberra. The Brown's River virus also retained its infectivity *in vitro* at higher temperatures and for longer periods than is recorded for virus Y, but parallel experiments with virus Y under our conditions gave similar high values.

TABLE 2.—*Dilution of the Brown's River virus. Number of plants, of eight inoculated, infected at the dilutions shown*

Experiment	Dilution					
	Undil.	5	100	1000	10,000	100,000
1	8		8	8	4	0
2				8	4	0
3		8	6	1	0	
4[a]		0	2	1	0	

[a] Tobacco leaves from the same lot of plants as in Experiment 3 were frozen for five days, ground up with a small quantity of a 50 per cent solution of K_2HPO_4 and the juice was expressed through muslin.

Heat Inactivation

Juice was obtained from infected plants of *Nicotiana glutinosa* or tobacco, diluted 1 in 5, and divided into 10-cc. lots, which were held for 10 min. at different temperatures. Tests were made both on untreated juice and on juice that had been clarified by spinning in a centrifuge, and buffered at 0.05 molar and pH 7 with phosphate solution. Infections were obtained from inocula that had been heated to 58° and 60° C., but not to 61° C. or higher temperatures.

Aging in vitro

Three experiments were performed by storing plant juice diluted 1 in 5 at 25° C., and inoculating at intervals to tobacco or *Nicotiana glutinosa*. In the first two experiments, the juice expressed from the diseased leaves was merely filtered through muslin. In the third, the inoculum was clarified and buffered with phosphate solution. Infections were obtained from inocula that had been kept for 10 or 11 days, but not for 16. In addition, virus was held at a few degrees above freezing point for more than a week without any noticeable diminution of infectivity.

IMMUNITY TESTS AGAINST VIRUS Y

A number of trials was made using tobacco and *Nicotiana glutinosa*, in which first virus Y and later the Brown's River virus were inoculated to the same plants. There were controls in all experiments inoculated singly with

virus Y and the Brown's River virus. The likeness of the symptoms produced by the two viruses, particularly on tobacco, rendered these experiments of relatively small value. However, where the strain of virus Y was sufficiently mild, there was an observable difference in the severity of symptoms produced by parallel inoculations on *Nicotiana glutinosa*. Using such a strain, some indications were obtained that it induced an immunity against subsequent infection with the Brown's River virus.

The most definite results were obtained with President potato systemically infected with virus Y. In some instances, full protection was afforded against the Brown's River virus. There was no necrotic reaction on inoculated leaves, whereas control President plants free from virus Y produced fully necrotic symptoms. However, some necrosis developed on the inoculated leaves of several other Y-infected plants after inoculation with the Brown's River virus. The necrosis was more diffuse than that usually produced at the site of inoculation on healthy plants, and may have been due in part to the original infection. There is no doubt that virus Y produced partial or complete immunity in President potato against subsequent infection with the Brown's River virus.

A few Y-infected plants of other varieties gave similar results when inoculated with the Brown's River virus.

DISCUSSION

The results of transmission tests and property studies leave little doubt that the Brown's River virus is virus C, and that virus C is a strain of virus Y (3, 4). The two instances of transmission by *Myzus persicae* suggest that virus C might occasionally be transmitted by this insect in the field. Several examples of field transmission under conditions of heavy aphid infestation (5) might be interpreted as rare examples of transmission by the normal vectors of virus Y.

Other examples of field transmission of what is possibly virus C are reported from New Zealand by Chamberlain (4). A virus complex carried in a potato variety, Aucklander Short-top, gave symptoms on a range of hosts that suggest it consisted of a mixture of virus X and B and another virus that causes top necrosis on Up-to-Date. In Chamberlain's greenhouse trials, no part of the virus complex was transmitted by *Myzus persicae* or *Macrosiphum solani* to healthy potatoes. Yet, "a severe top necrosis of several potato varieties was observed" in the field, and "the incidence of this disease was greatest in those lines which, in the previous season, had been grown in close proximity to the variety, Aucklander Short-top." Chamberlain's results are consistent with the view that the virus causing the top necrosis was virus C.

The chances of virus C causing serious economic losses are very small, except, possibly, in a new carrier variety that becomes infected as a seedling, or during the earliest stages of multiplication. From the incidence of the disease, it appears that this has been the mode of its perpetuation. Virus C

is seldom found except in carrier varieties, and, where it is found, it infects the whole or the greater portion of existing stocks. As an unusual variant of virus Y, it is of interest to students of virus diseases, but it is of no importance to the farmer.

SUMMARY

A virus isolated from an old Australian variety of potato is described, and identified with virus C. Its physical properties and host range, which are described, ally it closely with virus Y, but, except possibly in rare instances, the virus is not transmissible by the most efficient vector of virus Y, the aphid, *Myzus persicae*. The virus is unlikely to become widespread or produce serious losses in potato crops.

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TOBACCO ANTHRACNOSE, A PLANT BED AND FIELD DISEASE[1]

E. A. Walker[2] and Elizabeth Wise McInroy[3]

(Accepted for publication February 15, 1945)

Tobacco anthracnose in Maryland was first observed in a commercial Medium Broadleaf tobacco plant bed on May 24, 1941, near Waldorf in Charles County. A leaf spot was found in plant beds treated for the control of tobacco blue mold with 1½ pounds of paradichlorobenzene to 100 square yards of plant bed per treatment. This conspicuous leaf spot was distinctly different from any injury previously experienced when tobacco plants were treated with this gas-producing substance. The spots were small, circular, and scattered over the leaf, resembling to some extent the injury produced when nitrate of soda granules are in contact with the leaves after top-dressing with this fertilizer. Plants in untreated plant beds adjacent to those receiving the paradichlorobenzene gas treatment also had leaf spot. These plant beds were established in "new land" and were surrounded by a wooded area to the north and west, and sloped eastward toward a weed strip used the previous season for tobacco plant beds.

Many tobacco plant beds were then visited in Charles, Prince Georges, and Calvert Counties, and this same leaf spot was found distributed quite generally in Southern Maryland. In several tobacco plant beds seedling blight was observed in small areas where many plants had died. These were surrounded by plants with leaf spots like those first observed in Charles County. A similar leaf spot was found in the College tobacco greenhouse where 64 strains of Maryland type tobacco were bedded. It was so destructive as a seedling blight by the first of June, that only a few live plants remained in some seed plots.

Symptoms on tobacco plants are distinct. Young spots are small, light green, slightly water-soaked, and considerably depressed. These pin-point depressions soon enlarge to form circular spots $\frac{1}{16}$ to $\frac{1}{8}$ inch in diameter. As the spots dry out they become papery thin and grayish-white, and are surrounded by a raised water-soaked border, which later becomes brownish. Larger spots may become zonated and have a darker center. Many small lateral veins are killed and turn brownish-black. This causes the affected leaf to become wrinkled and distorted, and a portion of it may die (Fig. 1, A). If spots are numerous there may be a general breakdown and death of the entire leaf. Small plants the size of a quarter or half dollar, when severely affected are stunted or killed, resulting in large barren spots in tobacco plant beds.

[1] Scientific Paper No. A76, Contribution No. 1931, Maryland Agricultural Experiment Station (Department of Botany).

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Plants large enough for transplanting had small cankers on the leaf midrib and petiole. These lesions are water-soaked at first, and later become sunken to form oblong greenish-brown lesions ¼ to ½ inch long. Similar lesions, some circular and others oblong, were on the main stem (Fig. 1, B). These weaken the stem so that it breaks when plants are drawn for transplanting. Pink masses of spores surrounded by black tapering setae were in the lesions.

Isolations were made on 3 per cent malt agar, and a *Colletotrichum* was consistently isolated from plants with stem, petiole, and midrib lesions. The same organism was isolated from the leaf spots, which usually had few spores but numerous setae. A spore suspension was made and fresh tobacco seedlings were inoculated by spraying this ten-day-old, malt-agar spore suspen-

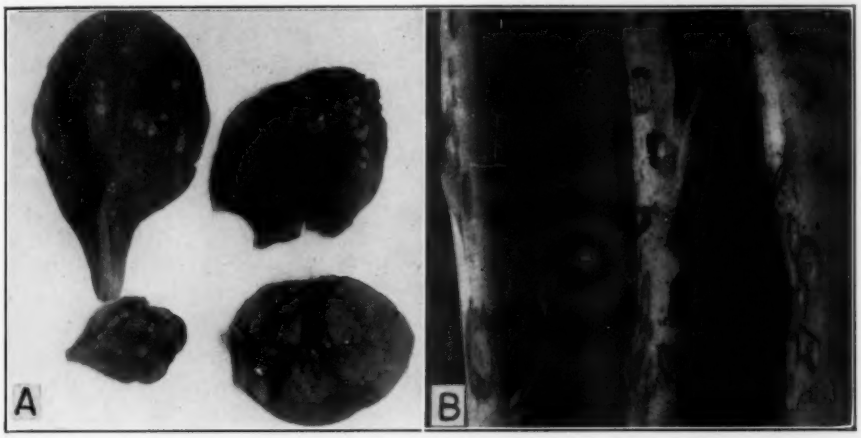

FIG. 1. Anthracnose on tobacco leaves and stems. A. Leaves of seedling tobacco plants with large anthracnose spots. The lower right leaf shows necrosis of the midrib and lateral veins. ×½ approx. B. Elongated anthracnose lesions on stems of tobacco seedlings. The stem to the right is practically girdled by the fungus. ×1 approx.

sion on the leaves. After these plants had been in a moist dark chamber for 60 hours, the inoculated leaves were severely spotted, and some plants were killed. The *Colletotrichum* was recovered, and reinoculated onto other healthy tobacco plant leaves, on which anthracnose spots developed, and the same organism was again recovered. This was repeated again to establish its pathogenicity for tobacco. The tobacco plants used were of the Maryland Medium Broadleaf type generally grown in Maryland commercial plantings. Similar tests were made on numerous wild and cultivated host plants by the detached leaf and potted plant methods, and the fungus was pathogenic to pokeweed, geranium, begonia, tomato, and potato leaves.

Numerous isolations were made from tobacco anthracnose stem, petiole, and midrib lesions and leaf spots during the first season. *Colletotrichum* sp. was isolated from most of the diseased material, from which single spore or hyphal tip cultures were obtained for further laboratory cultural studies. *Gloeosporium* sp. was isolated directly from some tobacco anthracnose

lesions and other strains of *Gloeosporium* sp. were obtained from dark sectors in cultures of *Colletotrichum*. Sectoring was abundant on cultures of the *Colletotrichum;* and spiral growth was observed in two strains of *Colletotrichum* and in two of *Gloeosporium*.

Tobacco anthracnose was first recorded from commercial tobacco plant beds in the United States in 1941. Johnson and Valleau (6) observed a similar disease on tobacco plants in the Station beds in Kentucky in 1935, and they called the organism *Colletotrichum destructivum*. Tobacco anthracnose was first observed in Brazil by Averna-Sacca (1, pp. 221–223),

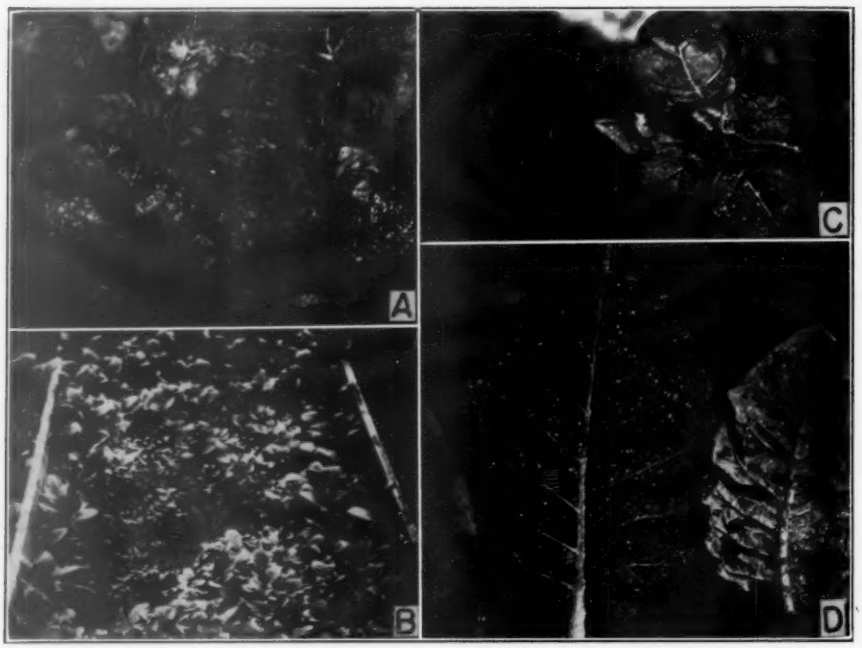

FIG. 2. Tobacco plants affected with anthracnose in plant beds, and infected leaves from plants grown in the field. A. Diseased plants with white circular leaf spots on lower leaves, and young upper leaves crinkled and distorted by anthracnose cankers on the midrib and lateral veins. B. Plant bed with plants killed by anthracnose while very small. Adjacent plants are small and dwarfed. C. Single plant from plant bed A showing leaf spots and crinkled bud leaves. $\times \frac{1}{2}$ approx. D. Middle leaves taken from field plants with small circular anthracnose spots. The leaf on the right has elongated young anthracnose cankers on the midrib. $\times \frac{1}{3}$ approx.

who studied the disease and named the causal organism *Colletotrichum nicotianae*. Böning (2, 3) in 1929 isolated and described the organism causing tobacco anthracnose in Germany as *Colletotrichum tabacum*. In 1932 Böning (4) isolated a similar organism, devoid of setae, to which the name *Gloeosporium* Desmaz. et Mont. was given. Tobacco anthracnose has been reported each year from Maryland since first observed in 1941 (9 through 15),[4] and from Pennsylvania in 1942 (5). The organisms obtained from

[4] Type specimens have been deposited in the Mycological Collection of the Bureau of Plant Industry, U. S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland.

Maryland tobacco anthracnose are being designated as *Colletotrichum* sp. and *Gloeosporium* sp.

During 1942 tobacco anthracnose became very severe in plant beds by May 18 in Prince Georges County (Fig. 2). A week later it was observed at one location near Gambrills, Anne Arundel County, where it had destroyed about 2,100 square yards of tobacco bed. The disease was very destructive in most plant beds visited in Southern Maryland. The plant beds at the Marlboro sub-station were infected, causing some loss from death of plants. Many diseased plants were transplanted to the field where the anthracnose disease continued to develop as leaf spots, leaf midrib and petiole cankers, and as stem cankers on the maturing plants. If the plants were set in very wet soil the disease developed rapidly on the new leaves, dwarfed the plants, and distorted the leaves. Cankers were also observed on the branches of the seed head and on the seed pods. In some fields anthracnose was observed on the "sucker" plants that developed after the crop was harvested. It is believed that the anthracnose organism is carried on the seed, and then carried to the fields on infected plants.

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CURLY-TOP AND CALIFORNIA-ASTER-YELLOWS DISEASES OF FLAX

HENRY H. P. SEVERIN AND BYRON R. HOUSTON

(Accepted for publication February 15, 1945)

Flax, *Linum usitatissimum* L., was reported to be susceptible to the curly-top virus by Severin[1] in 1929 and the symptoms on experimentally infected plants were described. No naturally infected plants were found prior to March, 1944, at which time the disease was generally distributed throughout the flax-growing region of Central California, most of which is located in western Fresno County. The estimated infection varied from 0 in the late planted fields to 5 per cent in the early plantings.

RELATION BETWEEN PLANTING DATE AND INFECTION PERCENTAGES

There was a definite relation between time of planting and infection percentages. The fields planted in October and November, 1943, were more severely damaged than those planted at a later date. The beet leafhopper, *Eutettix tenellus* (Baker), is normally present in large numbers on native and introduced weeds in the floor of the valley during the summer and autumn months. Severin[2] has stated that the autumn dispersal from these weeds begins in October and continues through November. During this period in 1943 there was a shortage of rainfall, thus limiting the feeding areas of the leafhoppers to cultivated crops, the principal one of which was early planted flax. By the time the flax that had been planted after November had reached the seedling stage, most of the leafhoppers probably had flown to the overwintering regions in the foothills of the Inner Coast Range bounding the west side of the San Joaquin Valley. This normal autumn flight of the vector probably accounted for the failure of the disease to become established in the later plantings.

SYMPTOMS IN THE FIELD

The first symptom noticeable in small seedling flax is the production of irregularly shaped wavy leaves closely grouped at the growing point (Fig. 1). There is then a gradual yellowing of the entire plant followed by death in most cases. Plants 8 to 10 inches tall when infected often continue to grow and produce the described leaf symptoms and a coiling of the tip of the stem (Fig. 1). Subsequent branches from such plants are often bent away from their normal position resulting in a spreading rather than an upright growing habit.

The most striking symptoms are those in the flowers. Diseased plants which reach the flowering stage produce small and much shortened inflores-

[1] Severin, Henry H. P. Additional host plants of curly-top. Hilgardia **3**: 595–629. 1929.

[2] Severin, Henry H. P. Field observations on the beet leafhopper, *Eutettix tenellus*, in California. Hilgardia **7**: 281–350. 1933.

cences. The petals of the flowers emerge from the calyx but remain closely pressed together and do not expand and open as do the normal flowers (Fig. 2, A, B). The petals are thus held until dry at which time they fall away from the ovary in a group. The diseased petals are twisted and puckered by small blister-like elevations over their entire surface (Fig. 2, D). Little seed is produced by infected plants.

FIG. 1. Disease symptoms on flax plants naturally infected by the curly-top virus. The stem on the left has the distorted and coiled tip with closely clustered malformed leaves often found on infected plants. The plant on the right shows the spreading rather than normal upright branching habit. Distorted wavy leaves are near the top of the upper stem.

Nearly all infected plants have a marked orange-yellow discoloration in the region of the phloem in the tap root and crown.

RECOVERY OF VIRUS

The virus was recovered from naturally infected flax by previously non-infective beet leafhoppers and transferred to healthy sugar beets which developed typical symptoms of curly-top.

DISCUSSION

It is probable that with the present trend to earlier fall planting of flax there will be considerable curly-top infection in these fields. Leafhopper infestation in the fall of 1943 was such that no large losses resulted. However, early plantings adjacent to areas abounding with preferred food and breeding plants such as Russian thistle (*Salsola kali* L. var. *tenuifolia* G. F.

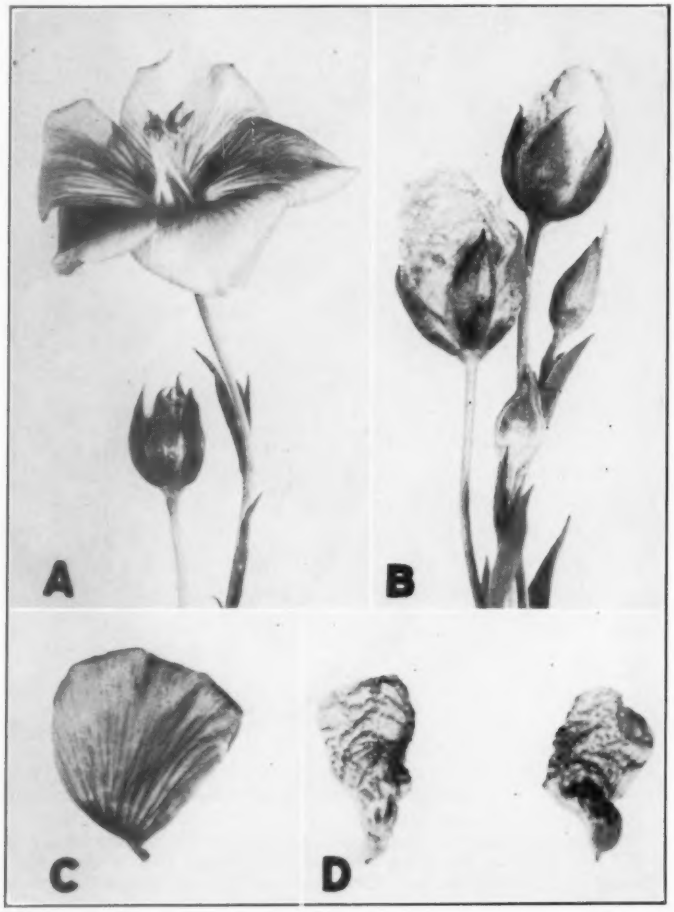

FIG. 2. Flax flower symptoms induced by the curly-top virus. A. Normal flax flower fully opened. B. Flowers same age as in A from diseased plant showing failure of bud to open. The entire inflorescence usually is in this condition. C. Petal from normal flower. D. Petals from diseased flower showing twisting and puckering in blister-like elevations.

W. Mey), bractscale (*Atriplex bracteosa* Wats.), and fogweed or silverscale (*A. argentea* subsp. *expansa* Wats.), which are host plants to both vector and virus, may easily suffer severe losses in years favorable to high leafhopper population.

FIG. 3. Flax experimentally infected by the California-aster-yellows virus showing secondary shoots arising from the axils of the leaves.

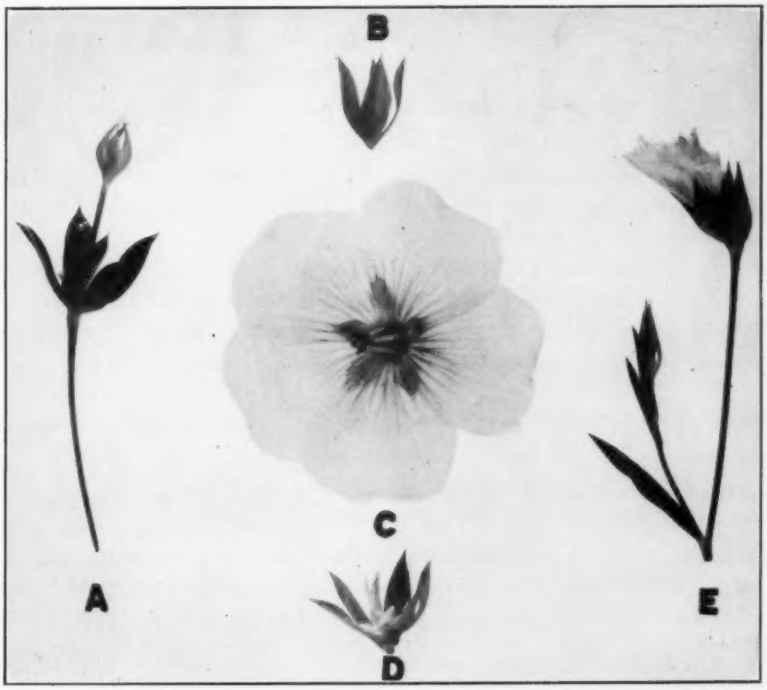

FIG. 4. Flax flower symptoms induced by the California-aster-yellows virus. A. Proliferation of flower. B. Flower with petals absent. C. Normal flower from control plant. D. Flower with reduced petals. E. Virescence or greening of flowers.

CALIFORNIA-ASTER-YELLOWS OF FLAX

Flax grown from seeds was experimentally infected with the California-aster-yellows virus by means of short-winged aster leafhopper, *Macrosteles divisus* (Uhl.), and the long-winged aster leafhopper, a race of the same species. The short-winged aster leafhopper failed to complete the nymphal stages, but a low population of long-winged adults was reared on flax.

The first symptom on experimentally infected flax is a yellowing of the apical leaves of the stems. Secondary shoots arise from the axils of the leaves (Fig. 3). Virescence or greening (Fig. 4, E) and proliferation of the flowers occur (Fig 4, A). The petals are often reduced (Fig. 4, C) or absent (Fig. 4, B).

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TRANSMISSION OF PEACH WART TO SWEET CHERRY[1]

S. M. Zeller and J. A. Milbrath

(Accepted for publication February 15, 1945)

Peach wart has become known in the Pacific Northwest through the work of Blodgett[2] who first observed the disease in 1938. He reported the disease in eastern Oregon but it has now been found as far west as Yamhill County in this State.

Two Improved Elberta peach trees in Yamhill County, Oregon, with the wart disease have been the source of inoculum of the transmission studies reported here. The disease was transmitted by budding five trees of each of seven varieties of peaches, Early Crawford, Early Muir, J. H. Hale, Improved Elberta, Orange Cling, Rio Oso Gem, and Rochester. All inoculated trees bore warty fruits the following season. There were no leaf or stem symptoms, which would indicate that the source of inoculum was free of other viruses.

It has been our practice to index the stone fruit viruses on several hosts in the genus *Prunus*. In this connection the symptoms of peach wart on sweet cherry have proved of interest.

Five trees of the Napoleon (Royal Anne) variety and three trees each of the Lambert and Black Republican varieties were inoculated by budding. Trees inoculated in August begin to show symptoms by the next April and by May the symptoms are very characteristic in the new growth of the stems and in the newer leaves.

In the Napoleon variety the stem symptoms at first are limited to necrosis in the vascular ring. This necrosis as far as 12 to 15 inches back from the tips results in a general die-back (Fig. 1). Sometimes there is more than normal branching. In addition the internodes of the last few inches near the terminals of the several branches are shorter and considerably larger in diameter than normal. This condition and resulting "leafiness" of the terminals give a rosetted appearance. There is a tendency toward smaller, narrower leaves as a result of crowding in the rosettes.

The stem reaction in the Napoleon variety also occurs when the Black Republican and Lambert varieties of sweet cherry are inoculated with the peach-wart virus.

The mottling produced in the leaves of the Napoleon variety is more characteristic and distinct than that produced in Black Republican or Lambert varieties of cherry. The mottling seems to start with chlorosis bordering veinlets. This spreads out to form larger ring-like patterns or even somewhat rectangular bands or lines on either side of veins (Fig. 2). In May and June the symptoms are especially prominent on most of the

[1] Published as Technical Paper No. 450, with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

[2] Blodgett, Earle C. Peach wart. Phytopath. 33: 21–32. 1943.

young leaves. As the season progresses and the leaves mature the symptoms become less conspicuous, until finally in late August the mottling has disap-

FIG. 1. Die-back of sweet cherry caused by peach-wart virus.

peared in all of the matured leaves, even though they may have been mottled earlier.

There is a similarity between the mottling produced in cherry leaves

by peach-wart virus and that produced by mild-mottle-leaf virus. Warty fruits, however, are not produced when peach trees are inoculated with mild mottle leaf of cherry. Cherry trees with the mild-mottle-leaf disease likewise do not have the stem symptoms described for peach wart in cherry.

The peach-wart virus was also transmitted from peach to cherry and back to peach without apparent attenuation. Buds from Napoleon and Black Republican cherry trees with symptoms of peach wart were used as inoculum in Early Crawford peach trees. Whether the virus came from peach through Napoleon cherry or through Black Republican cherry, the Early Crawford peach trees bore fruit with wart symptoms the next season.

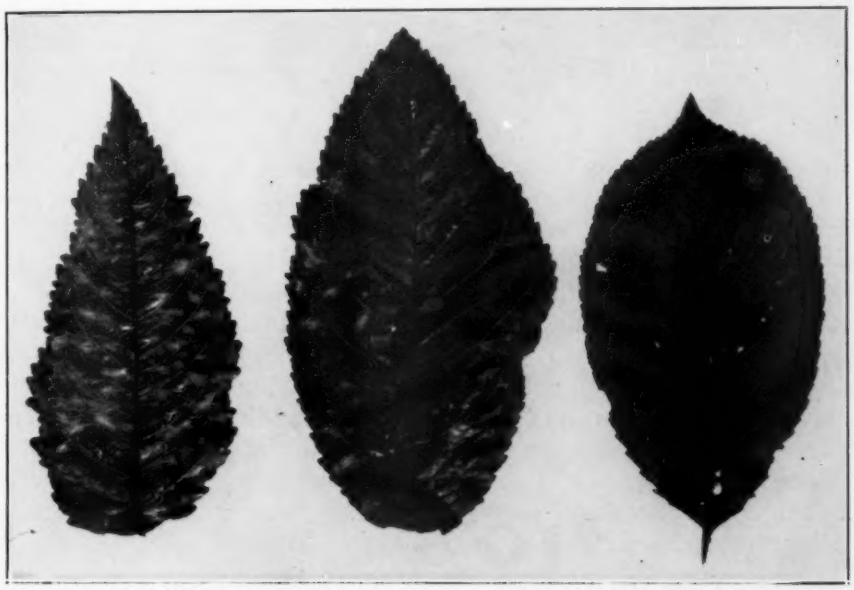

FIG. 2. Leaves of 3 stages of maturity from a Napoleon cherry tree inoculated with peach-wart virus. Young leaf with prominent mottling and two older leaves showing disappearance of mottling with maturity.

Cherry therefore may be a host of the peach-wart virus although this virus has not been found in cherry under natural conditions.

SUMMARY

Peach wart found in Improved Elberta trees in western Oregon was transmitted to seven varieties of peach trees and to Black Republican, Lambert, and Napoleon (Royal Anne) sweet cherry trees. The symptoms in sweet cherry leaves and stems are described. Peach-wart virus was also transmitted from the sweet cherry trees back to peach trees without apparent attenuation of the virus in cherry.

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ANATOMY OF BUCKSKIN-DISEASED PEACH AND CHERRY[1]

HENRY SCHNEIDER[2]

(Accepted for publication March 1, 1945)

INTRODUCTION

Anatomical changes induced by the presence of virus in plant tissues are of interest and importance for several reasons. They may serve to indicate the avenue of entry of virus into the plant and the subsequent path of migration. This may in turn be of assistance in determining the vector or vectors of a given virus. In many cases a knowledge of internal microscopic symptoms will lead to a better understanding of the external symptoms upon which diagnosis largely depends.

Necrosis of the sieve tubes is a characteristic anatomical change produced in buckskin-diseased peach on peach stock and in sweet cherry when on Mahaleb root stock. Rawlins and Thomas (16) noted that a disturbance occurred in the phloem of peach and cherry which was indicated by a wound-gum reaction when sections were treated with phloroglucinol and hydrochloric acid. They later stated that the phloem necrosis resembled that of potato leaf roll (21). Necrotic spots frequently form in the leaves of affected peach trees. The histology and histochemistry of the formation of these spots has been briefly described (16). The present paper considers the structure and development of the phloem of diseased peach and cherry and compares this tissue with the phloem of the healthy peach and cherry as described in a previous paper (17). Studies on the histochemistry of affected phloem tissues and on the anatomy of peach and cherry on incompatible root stock are also described.

EXTERNAL SYMPTOMS OF THE DISEASE IN PEACH AND CHERRY

Buckskin disease was first described and so named on the basis of material of sweet cherry (*Prunus avium* L.) collected at Green Valley, Solano County, California (14). The principal symptoms were failure of fruits to mature and the development of a leathery fruit surface. Leaves of infected cherry trees often became bright orange to red in the basal part of the lamina. Some years later a disease of peach at first designated as "leaf casting yellows" was also found to be caused by the buckskin virus (20). The chief symptoms in peach (*P. Persica* Sieb. and Zucc.) are yellowing and rolling of leaves, death and abscission of irregular areas in the lamina, and shedding of fruit and leaves of affected branches in early summer. Other symptoms

[1] Part of a thesis submitted in partial fulfillment of requirements for the degree of Doctor of Philosophy, University of California, 1943.

[2] The author is indebted to Drs. Katherine Esau and H. Earl Thomas for guidance in the work and preparation of the manuscript. Dr. Thomas and T. E. Rawlins supplied the plant material used. Several members of the Division of Plant Pathology of the University of California and the Guayule Research Project kindly read and criticized the manuscript.

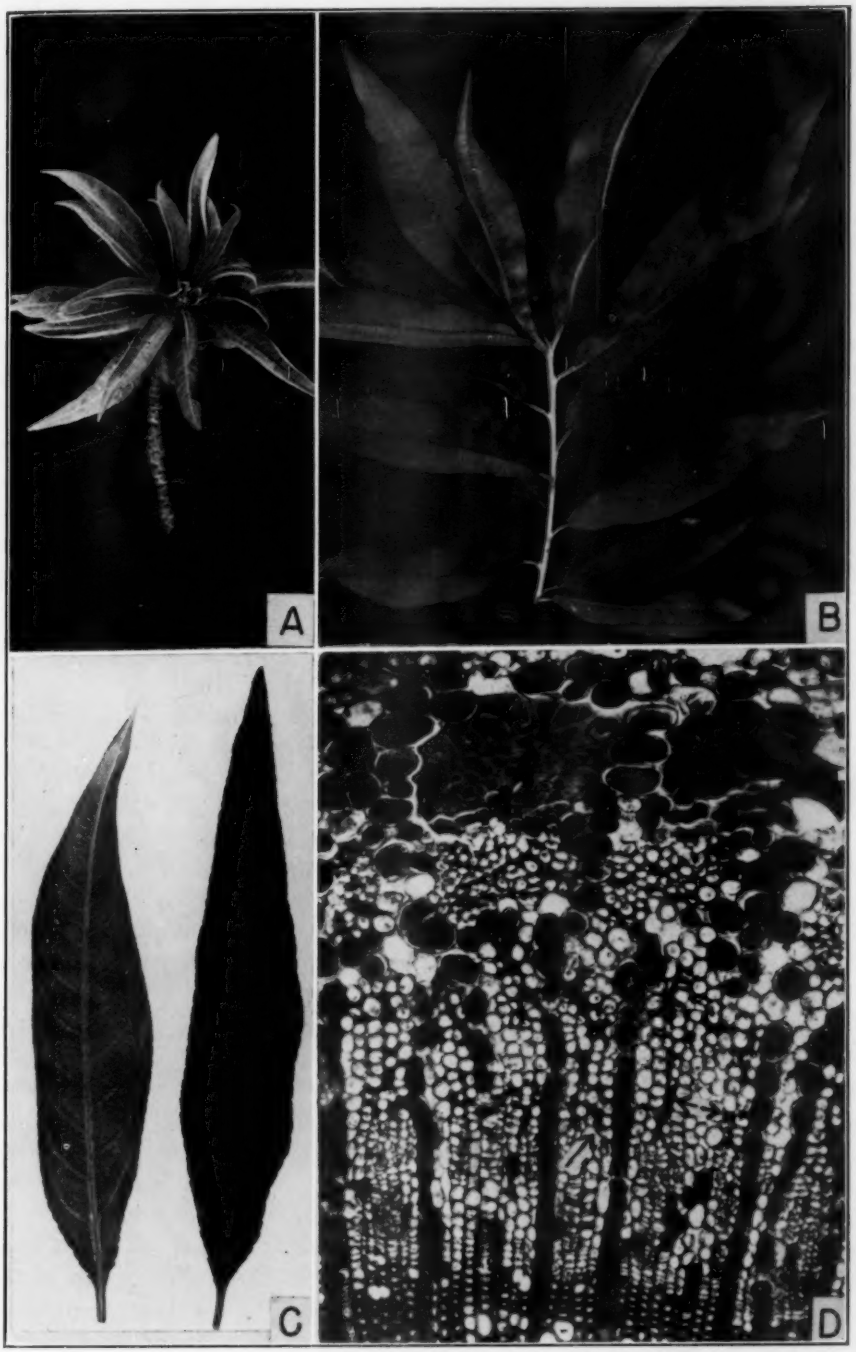

FIG. 1. A. Phillips Cling peach stem affected by the Palo Alto strain of the buckskin virus (×0.4). B. A healthy peach shoot (×0.4). C. Left. Swollen veins in a Muir seedling leaf affected by the Palo Alto strain of buckskin virus. Right. A normal leaf (×1.1). D. Transverse section of noninfected Palora peach stem (on Myrobalan stock) 1½ inches from the stem tip. Arrows point to three of the necrotic sieve tubes. In the new band of secondary phloem the sieve tubes have not undergone turgor expansion (×200).

produced by two distinct strains of buckskin virus on cherry and peach have been described in some detail (21).

Two new strains of buckskin virus[3] were used in some of the tests. One was collected in peach at Palo Alto, California. It has not been found in sweet cherry nor has it been successfully inoculated into a sufficient number of cherries to permit a detailed study of symptoms in them. The early symptoms produced by this strain in peach are similar to those produced by the Green Valley virus. These are followed, however, by extreme dwarfing of leaves and shortening of internodes (Fig. 1, A and B) while the terminal buds continue to grow slowly far beyond the normal growth period. Another strain of buckskin virus obtained from a peach orchard at Merced, California, produced symptoms similar to those caused by the strain of virus found at Green Valley, but the Phillips Cling variety of peach was more severely affected by it.

METHODS AND MATERIALS

The methods used on the diseased material are the same as those described elsewhere for healthy trees (17). Unless otherwise specified it is understood that the trees were inoculated with the Green Valley strain of virus. Special methods are given later for the work on diseased cherry.

ANATOMY OF DISEASED PEACH

Pathological Anatomy of the Primary and Early Secondary Phloem

In general the pathological changes observed in the primary and early secondary phloem of diseased peach trees are clear cut. At the onset of the disease the sieve tubes and companion cells become necrotic and collapse in the older part of the phloem (Fig. 2 and 3, B). In these early stages, the necrosis may then proceed even to the youngest sieve tubes (Fig. 4, B). Following this, new phloem may be formed to replace the affected phloem, and the sieve tubes in this new tissue may also become necrotic (Fig. 5, B, and 8, D). Although these are the usual features of the diseased phloem tissue, other characteristics have been noted in diseased stems and are described later with reference to the conditions under which they occurred. The structure of the median trace of leaf 13 from the shoot tip at a point just below where it diverges from the stem and above where it was first branched is used for comparison with healthy trees as described elsewhere (17).

In the greenhouse, young normal appearing shoots growing from the upper portion of diseased trees that had been cut back had no phloem necrosis or other abnormalities. In some cases such tips were found to be free of virus when they were grafted to healthy trees. Three weeks later symptoms were present on leaves two or three inches from the tips of the new stems on the trees that had been cut back. Sieve tubes of the primary phloem had become necrotic in the petioles of leaves with symptoms and a wide band of secondary phloem had been produced. In comparable normal petioles little secondary phloem is present and the metaphloem is not obliter-

[3] These strains were collected and tested and are here described by H. Earl Thomas.

FIG. 2. Transverse section of a young peach stem, newly affected by Green Valley buckskin virus. Some of the older sieve tubes of the primary phloem were obliterated and others were necrotic. The older sieve tubes in the secondary phloem were becoming necrotic (indicated by ×'s). Normal sieve tubes are marked with circles. The outer sieve tubes that have not collapsed do not have thick nacré walls (×575).

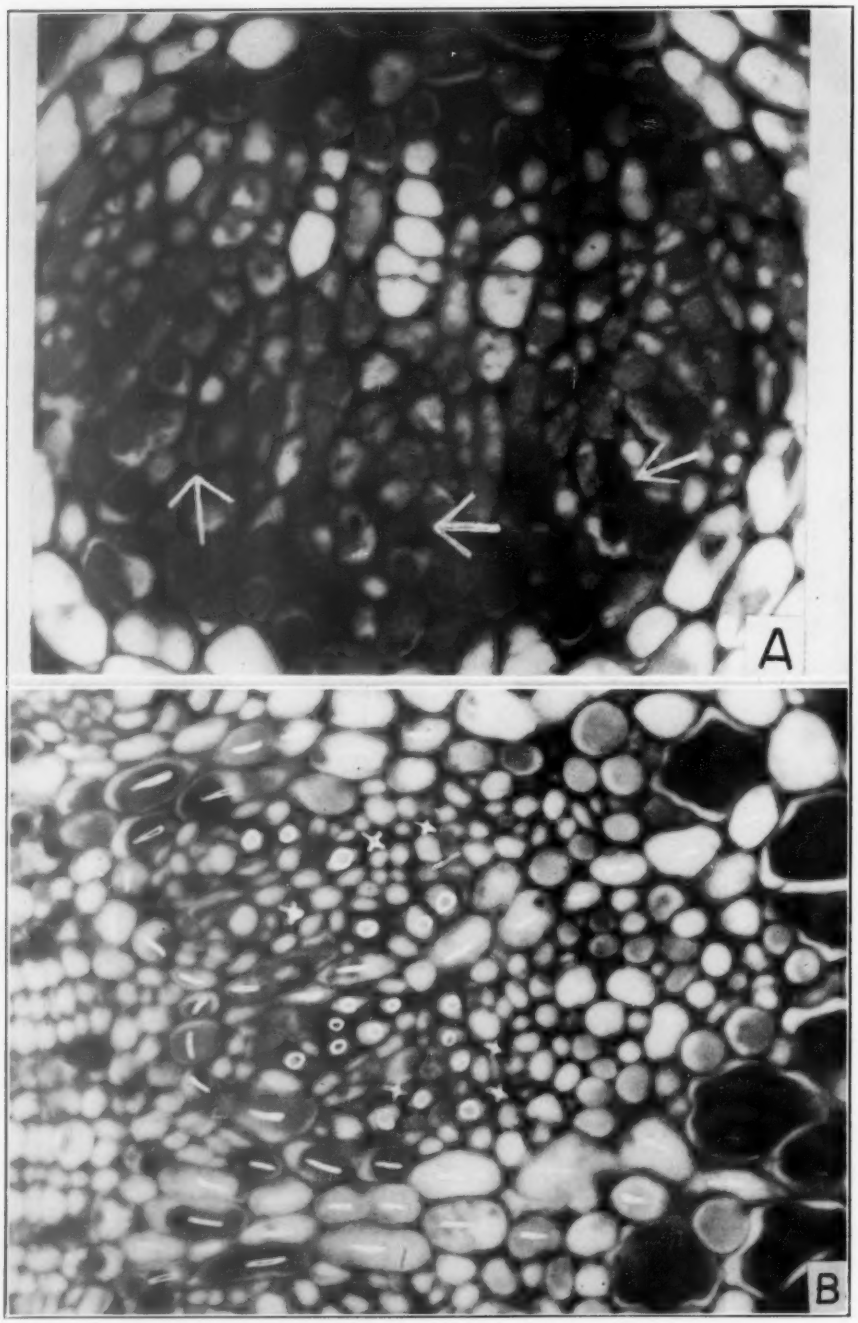

FIG. 3. Transverse sections from peach shoots affected by Green Valley buckskin virus. A. The median bundle of leaf base 7. Arrows point to necrotic protophloem sieve tubes (×1580). B. Phloem strands of the median trace from leaf 13 two mm. from the stem tip. The trace was not so profoundly affected as other traces at the same level. Several of the older sieve tubes are semi-collapsed (marked with ×'s). Normal sieve tubes are marked with circles. Dashes indicate the primary ray cells and the parenchyma cells between the primary phloem and the procambium (×590).

ated, even in old leaves. Necrosis of the metaphloem and older secondary sieve tubes in the stem below the affected leaves had also occurred (Fig. 2). Normally no secondary sieve tubes would be obliterated at this stage and some of the metaphloem sieve tubes would still be functioning. Sieve-tube necrosis was absent or just appearing in parts of the stem above the affected leaves.

Shoots from a tree affected by the virus for several weeks had ceased elongating and symptoms were present in leaves about five inches from the tips of the stems. Necrosis had occurred in many of the sieve tubes beginning with those first formed in the leaf primordia and stem (Fig. 3, A) and continuing with those derived from the cambium in parts of the stem producing secondary growth. The outer primary phloem fiber initials that had undergone turgor expansion but had not yet developed secondary walls, were occasionally collapsed in the leaf traces. The cambium in some places contained heavily staining precipitates. Its orderly arrangement was disturbed and cells were frequently crushed. In the median trace from leaf 13 of another of the shoots affected, for several weeks only a few more sieve tubes had collapsed under the influence of necrosis than would collapse in normal obliteration (Fig. 3, B). In some of the younger traces at the same level, however, all the sieve tubes had collapsed. Apparently the virus is not present in equal quantities or is not equally active in all leaves and traces. This is substantiated by the general experience that not all buds from affected shoots transmit the virus when transferred to healthy plants.

Another stem had severe leaf symptoms at its tip and appeared to have stopped growing as a result of the disease, but sections showed that a number of leaf primordia had been produced since the onset of the disease. Apparently all the sieve tubes in the stem at the time of the onset of the disease as well as some of the partially differentiated fibers were killed (Fig. 4, A and B). New imperfectly differentiated sieve tubes were present at the time the material was taken for sectioning. Parenchyma cells apparently produced after the onset of the disease had thin walls, wavy in outline. In the primary phloem of newly initiated traces, there was no differentiation of primary ray cells or of large fiber initials. A few recently formed phloem mother cells had divided to form sieve tubes and companion cells, but the sieve tubes did not undergo turgor expansion (Fig. 4, A). In tissues in and around the median trace of leaf 21, most of which was probably initiated before becoming affected by the virus, there were necrotic cells other than sieve tubes (Fig. 4, B). Some of the parenchyma cells were hypertrophied. All of the cells of the outer row of primary phloem fiber initials were collapsed. This type of flattening of the outer fiber cell initials was found in all traces at this level.

Some diseased shoots continue to grow after infection of branches (Fig. 1, A). The internodes of the new growth, however, do not elongate fully and the leaves are small and rolled. The veins soon become swollen (Fig. 1, C). The development of the phloem was somewhat abnormal in several such

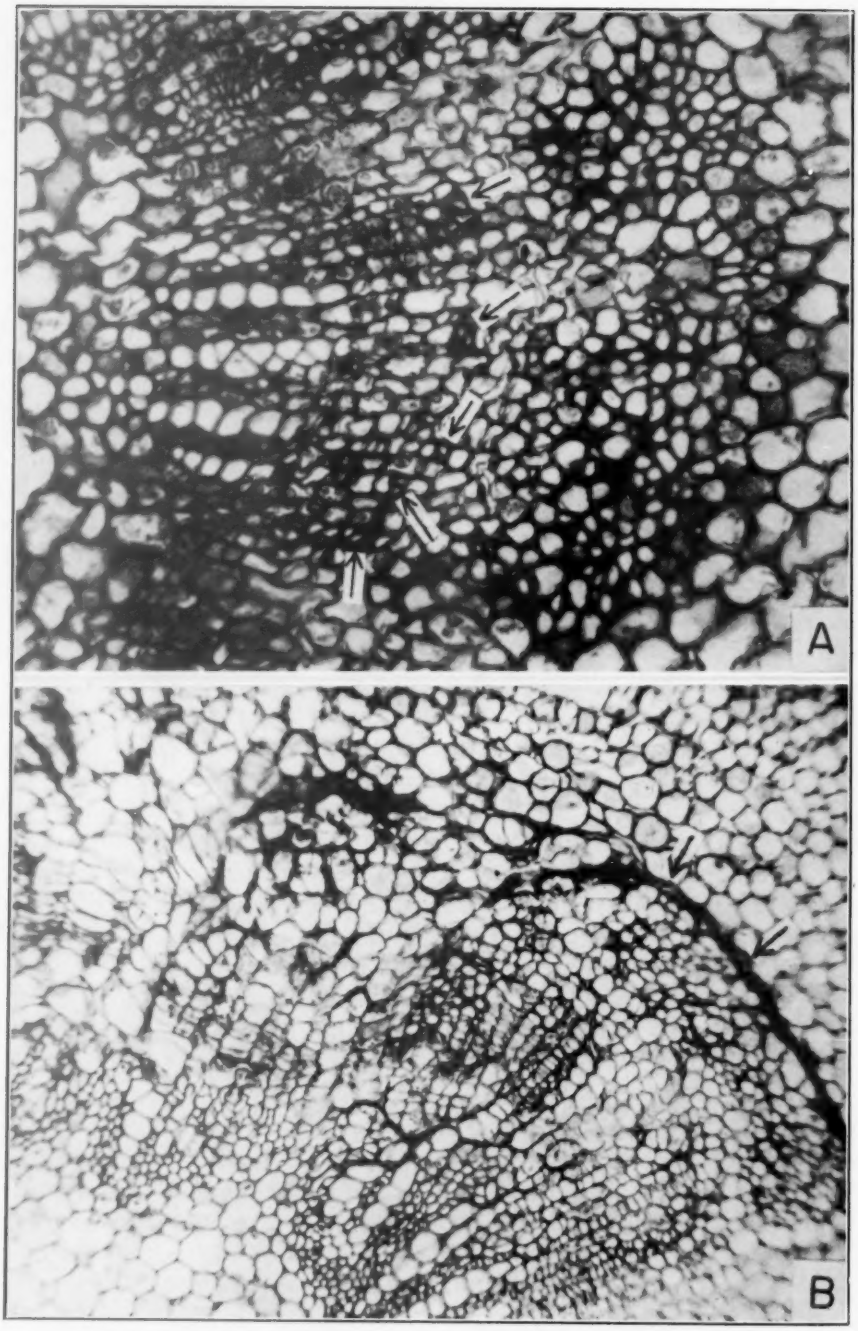

FIG. 4. Transverse sections of a peach stem affected by Green Valley buckskin virus. Some growth was produced after the onset of the disease. A. Median trace from the 13th leaf 0.8 mm. below the stem tip. Fiber initials failed to enlarge and the primary phloem did not form elliptical strands. The sieve tubes marked by arrows in the portion of the phloem produced most recently have not undergone turgor expansion after division of the phloem mother cells (×460). B. Median trace from the 21st leaf 11 mm. from the stem tip. Another branch trace appears to its left. Necrosis had occurred in the outer row of fiber initials (indicated by arrows), in the cortical parenchyma cells, sieve tubes, phloem, parenchyma cells, cambium, and xylem parenchyma cells. Some hypertrophied cells are present (×180).

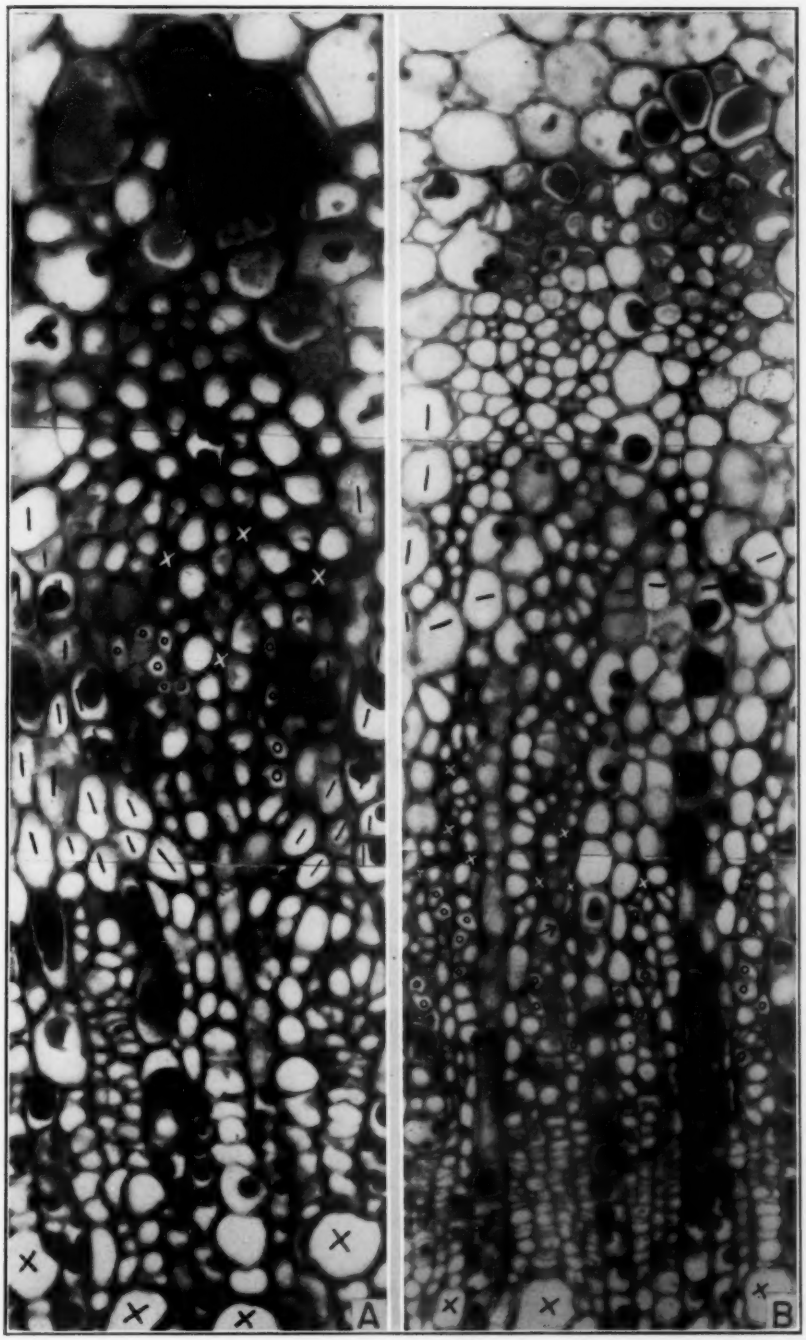

FIG. 5. Transverse sections of a peach stem chronically affected by Green Valley buckskin virus. A. Median trace from leaf 13 within the stem. Metaphloem sieve tubes do not have nacré walls. No secondary sieve tubes have differentiated in the secondary phloem. Otherwise the strand is normal. Details are: dashes, primary ray cells; ×'s, obliterated sieve tubes; circles, sieve tubes; ×'s in xylem are vessels (×570). B. A branch trace five mm. from the stem tip. Abnormal amounts of secondary phloem were produced and then obliterated abnormally soon. The arrow points to callus on a sieve plate. Dashes indicate the approximate boundary between the primary and secondary phloem; ×'s indicate necrotic sieve tubes bordering the region of normal ones; circles indicate sieve tubes; ×'s in the xylem indicate vessels (×400).

shoots. In one such shoot affected by the Green Valley strain of virus the youngest leaf primordia to contain sieve tubes developed apparently normally, but there was some indication that the older sieve tubes became necrotic sooner than they would have normally. The median trace of leaf 13 had developed very much like that of a normal stem: the primary phloem was laid down in elliptical strands that were more or less isolated from the secondary phloem by parenchyma cells; the fiber initials expanded normally; and about the usual number of sieve tubes were in the primary phloem (Fig. 5, A). It differed from the 13th trace of an elongating stem or one ceasing to elongate in that it was producing secondary phloem. However, in this

TABLE 1.—*Relative age of the leaf primordium with respect to differentiation of the vascular elements and necrosis of sieve tubes*

Stem tip number, nature of growth, and symptoms	Number of the leaf primordium in which character or change first appeared			
	Leaf base			Petiole
	Sieve tubes	Thick-walled xylem	Phloem obliteration or necrosis	Thick-walled xylem
1. Succulent, with apical growth. No symptoms	5	7	11	6
2. Apical growth nearly stopped. Symptoms on 5th expanded leaf. Newly infected	6	8	11	8
3. Apical growth stopping. Symptoms on 8th expanded leaf. Infection 7 weeks old	6	7	6[a]	.....
4. Same as 3	5	7	7[b]	6
5. Apical growth stopping. Symptoms on leaves at stem tip. Old infection	5	6	6[c]	6
6. Nodes short and leaves small. Chronic infection	6	7	7[d]	7

[a] Leaf bases 7 and 8 contained partially collapsed sieve tubes and leaf base 9 showed complete collapse of 5 out of 7 of them in some sections. (The degree to which a section of a sieve tube is collapsed depends on its proximity to a sieve plate.)

[b] Two sieve tubes crushed and one flattened (Fig. 3, A).

[c] All of the sieve tubes were collapsed in trace 7.

[d] Four out of five sieve tubes were crushed in trace 8.

respect it resembled a normal trace in a stem that had not produced apical growth for several weeks, except that no mature sieve tubes were in the secondary phloem of the diseased trace. Normally such maturation proceeds before so many cells are initiated. Nacré walls (thick crenulated walls with a pearly luster) were absent in the sieve tubes of the metaphloem. Fiber initials had not started to lay down secondary walls as occurred in the 13th median leaf trace of healthy Orange Cling peach stems which were growing slowly. At lower levels in the stem many more sieve tubes were necrotic than are normally obliterated (Fig. 5, B).

In summary it may be said that the most common anatomical change caused by the disease was the necrosis of sieve tubes. This necrosis was

similar to obliteration but occurred in sieve tubes before their normal period of functioning was completed. In severe cases the youngest sieve tubes became necrotic (Table 1), the orderly arrangement of cells in the cambium was affected, some parenchyma cells of the phloem and cortex collapsed while others were hypertrophied, and fiber initials in some cases were collapsed. Ontogeny after such severe attacks was abnormal. Primary rays did not develop nor did sieve tubes and fiber initials undergo turgor expansion. In stems that continued slow growth with shortened internodes and swollen leaf veins after arrival of the virus at the stem tip, ontogeny approached the normal; but the older sieve tubes became necrotic somewhat before they should normally have been obliterated and then they were replaced by new phloem.

Histochemical Changes in the Secondary Phloem

It has been reported that wound gum was present in the secondary phloem of peach affected by buckskin virus (16). According to Küster (11), wound gum is characterized by the following properties. It does not dissolve or swell in water, it is insoluble in alcohol, ether, carbondisulphide, potassium hydroxide, sulphuric acid, cold nitric acid, and cold aqua regia. It is soluble in warm nitric acid (concentration not given) and in a mixture of HCl and $KClO_3$. It stains red with phloroglucinol and HCl. Hewitt (9) in his studies on olive regarded wound gum as a water-insoluble substance which turns red when treated with phloroglucinol and hydrochloric acid but gives a negative reaction with the Maule test for lignin. The material here referred to as wound gum does not have all of the properties ascribed to it by Küster, but it does have the properties of the substance in olive abscission layers which Hewitt refers to as wound gum.

Wound gum was confined for the most part to the walls and contents of collapsed sieve tubes of diseased peach, but in some instances it extended into the middle lamella of adjacent parenchyma cells. Sieve tubes first lose their thick nacré walls and callus appears on the sieve plates. Later they collapse and wound gum may be observed. Sieve tubes may be completely flattened by the pressure of adjacent cells (Fig. 6, B and D). It was noted at times that not all of the necrotic sieve tubes contained wound gum; although, the time for its synthesis in the process of necrosis may have been passed. Wound gum was not present in the primary sieve tubes of peach.

The phloroglucinol-staining material in the walls of buckskin-diseased sieve tubes was not removed after sections cut in paraffin were treated in 10 per cent nitric acid in a test tube immersed in a bath of boiling water for three hours. However, it no longer reacted with phloroglucinol and HCl after this treatment. Wound gum was not completely removed from apple wood affected by *Polystictus versicolor* when subjected to the same treatment. It was not removed from the diseased sieve tubes of peach or from vessels of diseased apple wood after treatment in a mixture of equal parts of saturated $KClO_3$ and 18 per cent HCl for 18 hours. This mixture

yields chlorine gas as an end product. Sieve-tube walls of peach remain yellow when tests are made for cellulose with IKI and H_2SO_4 after these treatments. After removal of the wound gum by alternate treatments with

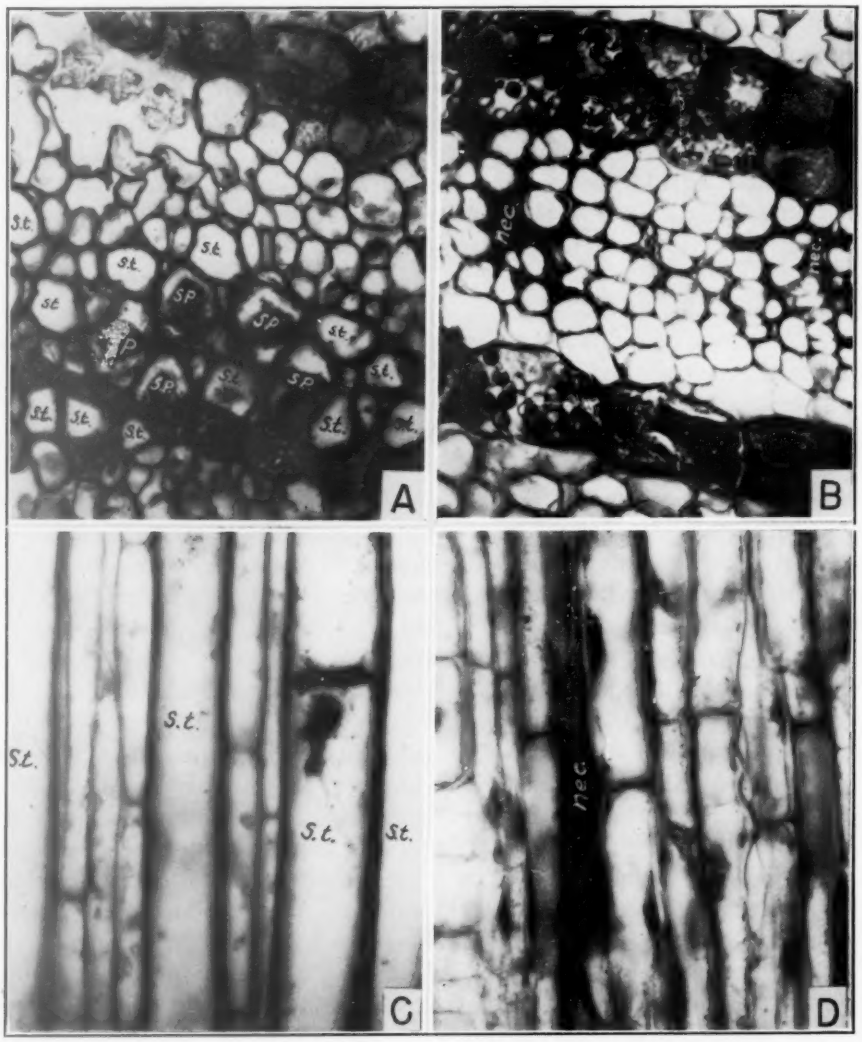

FIG. 6. Sections of secondary phloem of peach taken in December from greenhouse trees. A. Transverse section of healthy phloem between two rays. Sieve tubes have thick walls and sieve plates are in view in some of them. Calcium oxalate crystals have caused several parenchyma cell walls to disintegrate. B. Transverse section of buckskin-diseased phloem. All sieve tubes and companion cells have become necrotic. C. Longitudinal radial section of healthy phloem. One sieve plate is in view. D. Longitudinal radial section of buckskin-diseased phloem. One sieve tube and companion cell have degenerated. (A and B, × 465; C and D, × 640. s.p.—sieve plate; s.t.—sieve tube; nec.—necrosis.)

chlorine water and 2 per cent Na_2SO_3 at 95° C. (the method of Cross and Bevan for removing lignin) the affected walls stained much darker blue than

adjacent parenchyma cells when tested for cellulose with IKI and H_2SO_4. Occasionally weak coloration was obtained in affected sieve tubes when sections were treated by the method of Maule for lignin.

Stem Cankers

When a virulent strain of virus or a very susceptible peach variety is involved, rapidly growing shoots frequently develop cankers on the stem soon after the onset of disease. In Phillips Cling peaches affected by a strain of virus found in an orchard at Merced, California, this was particularly obvious. In the cortex of one such stem there was a large gum pocket and the cells around it from the epidermis to the xylem were necrotic (Fig. 7, A). Two smaller gum pockets were in the phloem and cambial region of the necrotic tissue. In the living tissue near the canker various abnormalities occurred. In the unlignified area of the xylem just below the cambium, gum ducts were forming. The cambium was abnormal in that it was not a layer of periclinally dividing rectangular cells. Instead, cells had divided in all directions and had become polygonal. In the most recently produced phloem the sieve tubes had not matured. In the older secondary phloem and in the primary phloem, the sieve tubes were necrotic. In another canker, a portion of the cortex and phloem were isolated by a periderm (Fig. 7, B). Primary phloem fiber strands were also isolated by periderm layers. Gum ducts were in the unlignified xylem tissue just beneath the cambium. In diseased material of a naturally infected seedling peach collected at Palo Alto, California, isolation of portions of the cortex and of the primary phloem fibers by periderms was very common. Gum ducts in the wood were frequently observed.

Anatomy of Swollen Veins in Peach Leaves Affected by the Buckskin Virus

Most leaves on peach branches affected by buckskin are prematurely abscissed. The veins of the older leaves that do not fall often become greatly enlarged when the plants are grown under glass (Fig. 1, C). Vein swelling is also seen occasionally in affected orchard trees. Rawlins and Thomas (16) reported that the swelling results from hypertrophy and hyperplasia in the phloem area; and that because of the vein-swelling the upper epidermis and palisade tissues separate from the lower epidermis and spongy parenchyma. Cavities arise on both sides of the vein. Cells of the bundle sheath frequently expand into the cavities and become quite large. The writers observed no necrosis.

In the present work swollen veins were characterized by necrotic sieve tubes and an abnormal production of secondary phloem. Occasionally secondary xylem has been observed in sections of swollen veins. Most of the phloem cells produced were parenchyma cells, rectangular when observed in a radial section. The sieve tubes were crushed and could be identified by the callus, stained with lacmoid, on their end walls.

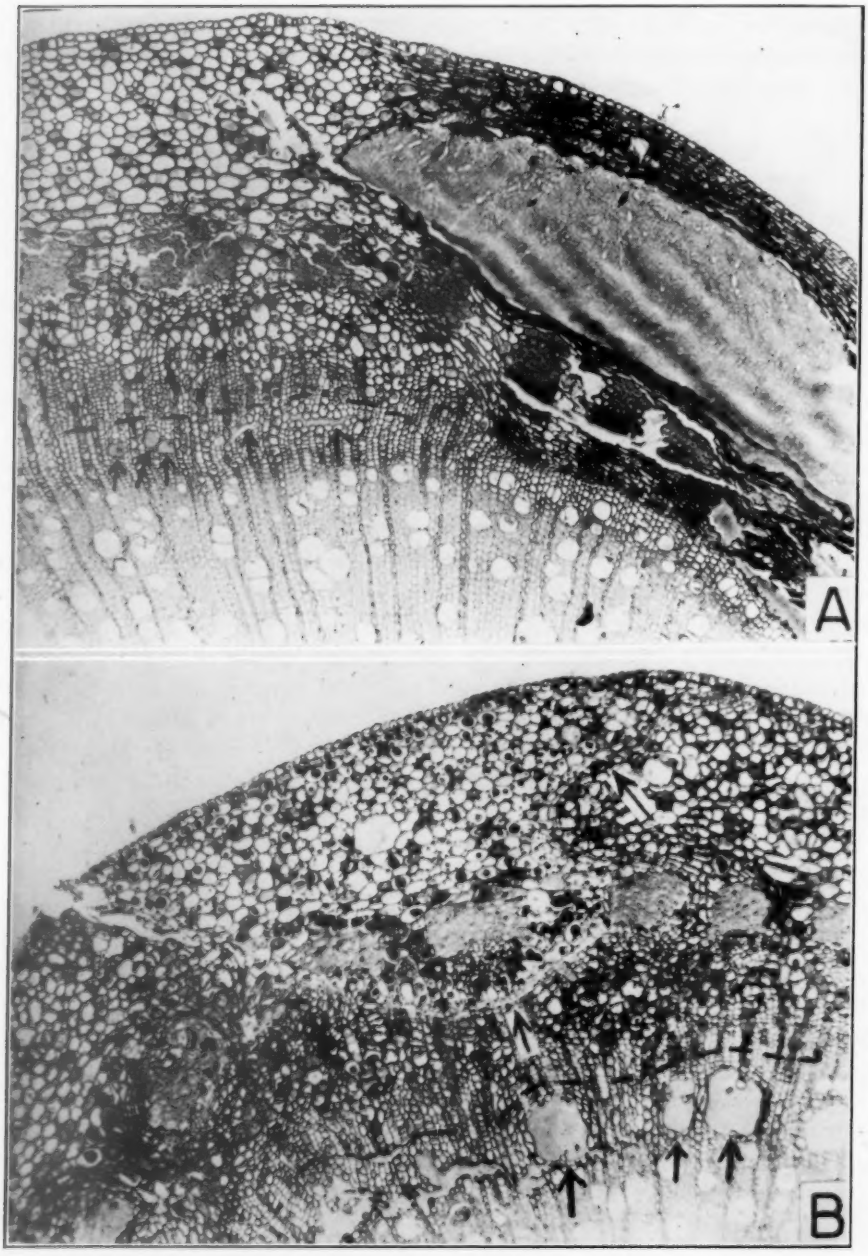

FIG. 7. Transverse sections of cankers in Phillips Cling peach stem affected by a strain of buckskin virus found at Merced, California. The position of the cambium is indicated by dashes. A. The canker on the right contains gum pockets and dead collapsed cells of tissues from the epidermis to the xylem. To the left of the canker, necrotic sieve tubes appear in the metaphloem and outer secondary phloem. There are gum pockets in the unlignified xylem (indicated by arrows). B. Another canker in which the dead area of the cortex and phloem is walled off by a periderm (indicated by arrows). A strand of primary phloem fibers to the left is also isolated by a periderm. Gum ducts in the xylem are indicated by arrows. Dashes mark the position of the vascular cambium. (Both $\times 95$.)

Affected petioles show a similar anatomical picture (Fig. 8). All of the primary phloem sieve tubes may become necrotic as well as the older abnormally produced secondary sieve tubes. Longitudinal sections of the abnormally produced secondary phloem show rectangular parenchyma cells with crushed sieve tubes between them. The presence of sieve plates in the end walls of the crushed sieve tubes has not been ascertained, but staining with lacmoid indicated that callus had developed.

Swollen Veins and Phloem Degeneration Caused by Ringing of Stems of Disease-Free Peach Trees

Leaves above an injured portion of stem may develop swollen veins. This suggested an experiment to determine if ringing would cause vein swelling similar to that in buckskin-diseased trees. A ring of bark about one-half inch wide was removed from each of several branches. The ring was wrapped with grafting tape to prevent drying. In an Orange Cling peach tree girdled in July the leaves eventually turned yellow, developed swollen veins, and many abscissed. Leaves of nonringed branches remained green, with normal veins. Sections of veins and petioles of leaves from ringed branches indicated that the cambium produced excessive amounts of secondary phloem. The newly produced sieve tubes resembled normal ones, and the original primary sieve tubes developed callus and were obliterated. Occasionally veins are very profoundly affected. In one such case cells of the xylem and phloem on one side of the vein became hypertrophied. The cambial region was very active and produced an abundance of flattened cells. Normally the metaphloem functions during the life of the leaf, with production of a trace of secondary phloem in petioles but none in lateral veins.

Two weeks after having been ringed the peach stems showed considerable change in the phloem in sections taken one-half inch above and below the ring. Leaves above the ring were still green and normal at this time. In the sections one-half inch below the ring vertical gum pockets had formed in the unlignified xylem mother cells just beneath the cambium. The cambium was not active and the sieve tubes still had thick nacré walls. No wound gum was present. Above the ring the sieve tubes showed a wound gum reaction of medium intensity, gum pockets had formed in the outer xylem, the cambium was very actively producing new xylem and phloem, and the older secondary sieve tubes were partially collapsed. Callus was observed in some of the sieve plates of the collapsed sieve tubes. Sections three and one-half inches below the ring were normal. In sections taken four inches above the ring the sieve tubes in the outer half of the secondary phloem were partially collapsed and their sieve plates were callused, but the nacré walls were still present. Phloem in sections thirteen inches above the ring were normal. Two nonringed branches were used as checks. Stem sections from immediately above the rings of branches ringed three months earlier revealed that the sieve tubes in about the outer three quarters of the phloem had lost their nacré walls and were collapsed. The functioning sieve

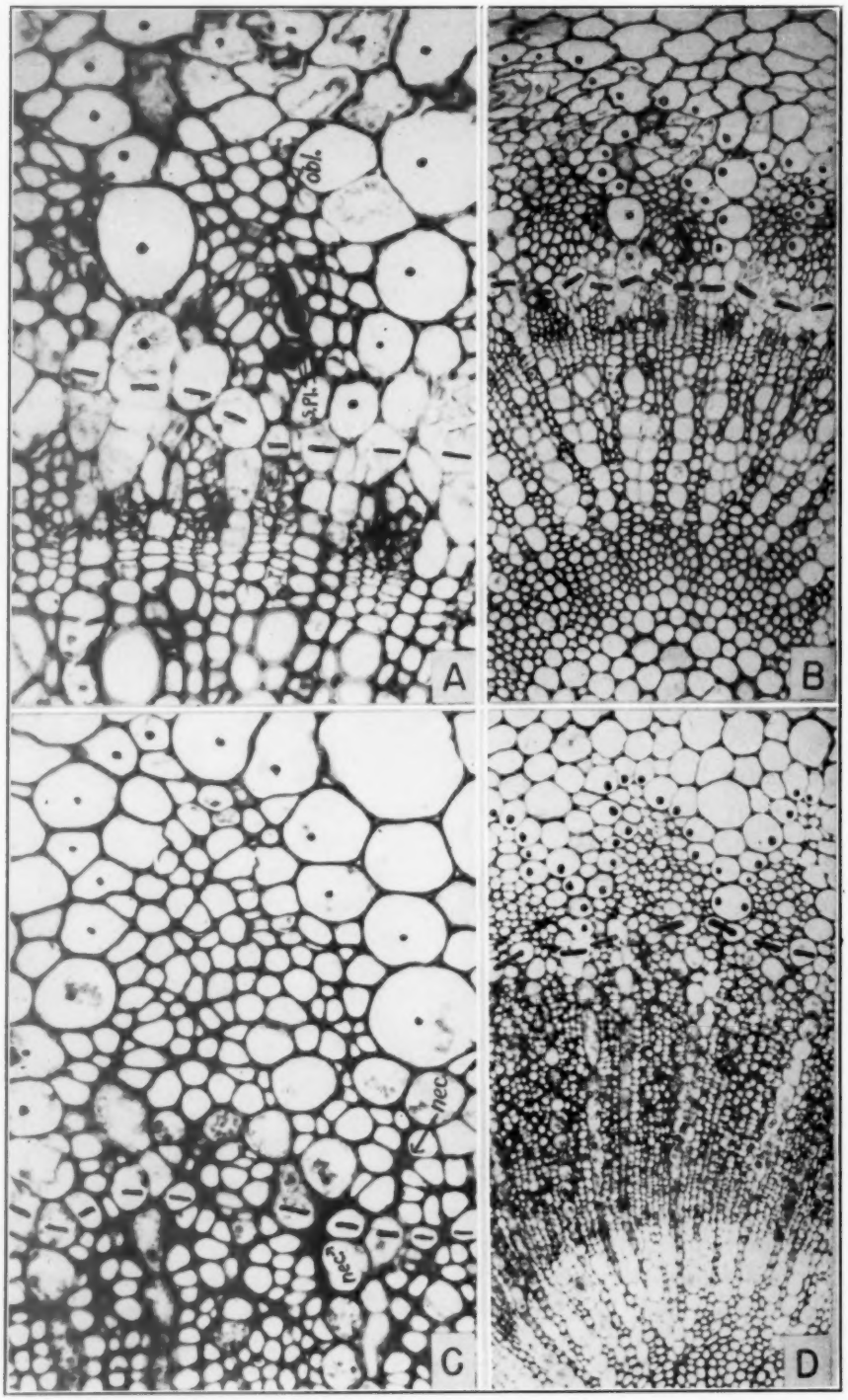

FIG. 8. Cross sections of peach leaf petioles. A and B healthy. (B lower magnification of A for orientation.) Dashes set off the primary phloem strands from the secon-

tubes in the quarter of the phloem near the cambium were small but otherwise appeared to be normal.

Phloem Degeneration in Healthy and Buckskin-Diseased Peach on Incompatible Rootstocks

Myrobalan (*Prunus cerasifera* Ehrh.) rootstocks are not completely compatible with peach tops. After peach scions or buds on such stocks grow to be several feet long under lath or in the greenhouse the leaves often roll, turn yellow, have swollen veins, and drop prematurely. Since these symptoms are similar to those of a buckskin-diseased peach, except that no death and abscission of irregular areas of the leaves occurs, the anatomy of such shoots is of interest. In several such stems a positive test for wound gum was obtained in the phloem. In a stained section of one such stem the sieve tubes of the metaphloem and the outer half of the secondary phloem were necrotic (Fig. 1, D). The inner part of the secondary phloem consisted of new tissue in which the sieve tubes were about half the size of the average parenchyma cells. The sieve tubes evidently had failed to enlarge after the division of the phloem mother cells.

No wound gum was observed immediately below buckskin-diseased peach scions (Merced and Palo Alto strains) in four Myrobalan trees or in the trunk or tap root of one of them. The peach scions had severe phloem injury.

ANATOMY OF BUCKSKIN-DISEASED CHERRY

Rawlins and Thomas (21) reported that wound gum is present in the phloem of stems of young buckskin-diseased sweet cherry tops on Mahaleb stock. When tops on Mahaleb stock become infected they die; however, such infections rarely occur naturally. The vegetative growth of trees on Mazzard roots on the other hand is not seriously affected by the disease (15). Examination of the phloem of these plants has suggested some explanation of these phenomena.

Methods and Materials

Necrosis of sieve tubes caused by buckskin virus in cherry was determined in many instances by a positive test for wound gum in the sieve tubes. Sliding microtome sections were used. The coloring by phloroglucinol and HCl due to the presence of wound gum was graded into three classes depending on its intensity and the area it covered. The location of the gum was

dary phloem of which only a trace is formed. The inner side of the primary phloem strands contains functioning metaphloem sieve tubes. The outer portion is composed of protophloem cells which enlarged and lengthened as the protophloem sieve tubes were obliterated. In stems, such cells have secondary walls and are the primary phloem fibers. s.pl.—sieve plates. obl.—obliterated cells. Dots in parenchyma cells adjoining primary phloem strands indicate the approximate division between the cortex and primary phloem. C. Buckskin-diseased petiole. The metaphloem sieve tubes are necrotic (outer margin of the metaphloem is indicated by dots in adjoining parenchyma cells). There are no large parenchyma cells separating the primary phloem from the secondary phloem. D. Lower magnification of C showing a wide band of secondary phloem which was produced and in which the sieve tubes became necrotic. Dashes indicate the approximate division between the primary and secondary phloem. nec. = necrosis. (A and C, × 330; B and D, × 125.)

also recorded in some cases. Lacmoid stain was used at times to determine the presence of definitive callus on the sieve plates. The presence of callus in amounts sufficient to completely fill the pores in the sieve plates was regarded as the first sign of cessation of functioning of the sieve tubes. Sections were made at several places on each tree. Celloidin and paraffin sections were also prepared from bark of several of the trees from an experimental field plot.

Wound Gum in Sieve Tubes of Healthy Cherry Trees with Mahaleb Root Stocks

To determine whether healthy trees contained wound gum the phloem of four young trees grown in the greenhouse was studied during the summer. A band of large sieve tubes formed during the spring and summer is here called the summer phloem, and the narrow band of smaller sieve tubes produced in the autumn is called winter phloem (17). Occasional sieve tubes of the old nonfunctioning phloem and outer summer phloem in a side branch of one tree, and a few sieve tubes of the main trunk of another contained wound gum. Sections through the main trunk above and below the bud union of one tree and through the tap root of another tree revealed no wound gum.

In addition, three noninoculated Napoleon cherry trees on Mahaleb stock which were dying from root rot were also sectioned through the trunk. One was free of wound gum and the other two contained occasional sieve tubes with gum in the outer border of the summer phloem and in the previous winter's phloem.

The trunk and older limbs of healthy Napoleon orchard trees with Mahaleb root stock showed wound gum more often than trees grown in the greenhouse. Most of the wound gum was formed in the autumn. At this time definitive callus formed on the sieve plates somewhat earlier in the Mahaleb stock than in the sweet-cherry tops. Such callus formation began in the older sieve tubes. This difference in callus formation was evident in some sections made about an inch above and below the graft unions of four Napoleon cherry scions on Mahaleb cherry stock. The branches were from four to six inches in diameter and were still in full leaf. The cessation of sieve-tube function in the outer half of the Mahaleb phloem probably caused a barrier to translocation in the outer half of the still active phloem of the Napoleon tops and played a part in the necrosis of sieve tubes in the Napoleon tops. There were usually more sieve tubes with wound gum above the union than below. The fact that the width of the summer phloem of Mahaleb stock in five orchard trees was only about five-sevenths of that of the Napoleon top is also of interest in this connection. Likewise the Mahaleb stock of a greenhouse tree had a band of summer sieve tubes 175 microns wide as compared to 290 microns for the Napoleon top in sections taken above and below the bud union. The ratio of parenchyma cells to sieve tubes in the two species appeared to be comparable but no detailed study

was made. In side branches of sweet cherry tops taken from the same orchard trees several feet from the unions there was little or no gumming of sieve tubes.

In sections from orchard collections made in the winter, three of ten side branches near the bases of trees gave mild wound-gum reactions, five contained occasional sieve tubes with wound gum, and one was free of wound gum. The mild reactions were observed 400 to 500 microns from the cambium.

In the summer, sieve tubes with wound gum were found in the phloem of the previous winter and early spring. Sections above the bud union from a healthy tree budded near the ground level contained a few sieve tubes with wound gum on the outer margin of the summer phloem, but no gum was present in a branch near the top of the tree. Two trees which were dying because of girdling by gophers did not contain gum in the summer phloem, but a small amount was in the previous winter's phloem. One section made above a bacterial gummosis canker on a large limb showed a mild reaction in the outer part of the summer phloem and a strong reaction occurred in the previous summer's phloem in the Mahaleb stock below the canker.

These observations show that wound gum is sometimes found in nonfunctioning winter phloem and in the outer summer phloem of apparently healthy orchard trees on Mahaleb stock. Apparently sweet cherry tops are not entirely compatible with Mahaleb stock. The slight incompatibility between the two cherry species appears to be caused by differences in phloem area and in differences in the time of callus formation in the autumn.

Wound Gum in Sieve Tubes of Buckskin-Diseased Trees on Mahaleb Stocks

Four Napoleon trees on Mahaleb rootstock, growing in a glasshouse and inoculated with Green Valley buckskin virus, were sectioned in different places in each tree just as symptoms were beginning to show. The results are illustrated in figure 9 which gives a diagrammatic longitudinal view of the phloem of one of the trees and is characteristic of all of them. The phloem with no wound gum in the Napoleon tops is of about the same width as the summer phloem of healthy trees, but only about 70 microns of unaffected phloem remains in the Mahaleb stock just below the bud union. The 70 microns include the cambium and partially differentiated sieve tubes. Only a few spots of wound gum were present ten inches below the union and no gum at all was present below a branch of the Mahaleb stock. The side branch of the Mahaleb stock did not contain wound gum. Seven branch roots at distances of eight to twelve inches from the bud unions of two of the other trees similarly affected at the graft unions either contained no gum or only occasional spots in the outer part of the phloem. The fourth tree gave a reaction of medium intensity in the only root sectioned. A tree which had lost all of its leaves because of the disease had wound gum in the sieve tubes of the phloem much nearer to the cambium in the Napoleon top than had been the case in the trees just beginning to show symptoms.

Tests have been made for wound gum in diseased orchard trees. Measurements of the phloem in four diseased Napoleon scions on forked Mahaleb stocks in the fall indicated that the region near the union contained two and one-half times the amount of summer phloem as did healthy Napoleon branches on the other fork of the same Mahaleb trees. The abnormal tissue contained more phloem parenchyma cells and fewer sieve tubes than normal summer phloem, and the sieve tubes in the older part were necrotic. The sieve tubes of the Mahaleb stock were necrotic nearer the cambium than were those of the Napoleon tops, and in two cases they were ultimately killed all the way to the cambium. Material collected at other times of the year has also

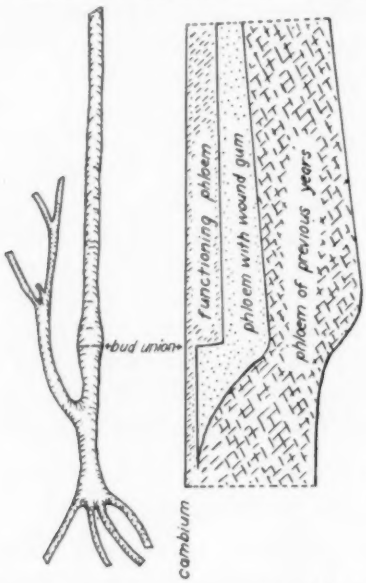

FIG. 9. Left. Sketch of a cherry tree on a Mahaleb cherry stock. The larger branch which was severely affected by the buckskin virus is the Napoleon cherry portion. The smaller Mahaleb branch did not contain wound gum in the phloem. Right. A diagram of the phloem of the right side of the tree showing the location of wound gum. Sieve tubes in most of the mature phloem of the Mahaleb had become necrotic.

shown that more phloem tissue is killed in the Mahaleb stock than in the Napoleon tops (Fig. 10).

Studies were made of the occurrence of wound gum at levels below unions of diseased orchard trees on Mahaleb stock. Injury was just as severe in portions of the stem nine to twelve inches below the graft union as immediately below the union in four branches of Mahaleb with severely affected Napoleon scions. In the case of a tree budded near the ground level the amount of injury decreased at increased distances below the union. The tree was just beginning to show leaf symptoms. At 38 inches below the union only occasional sieve tubes contained wound gum. Another tree, inoculated at the same time but taken six weeks later when the leaves were wilted and yellow, had uniform amounts of wound gum as far as twenty-two inches below the bud union.

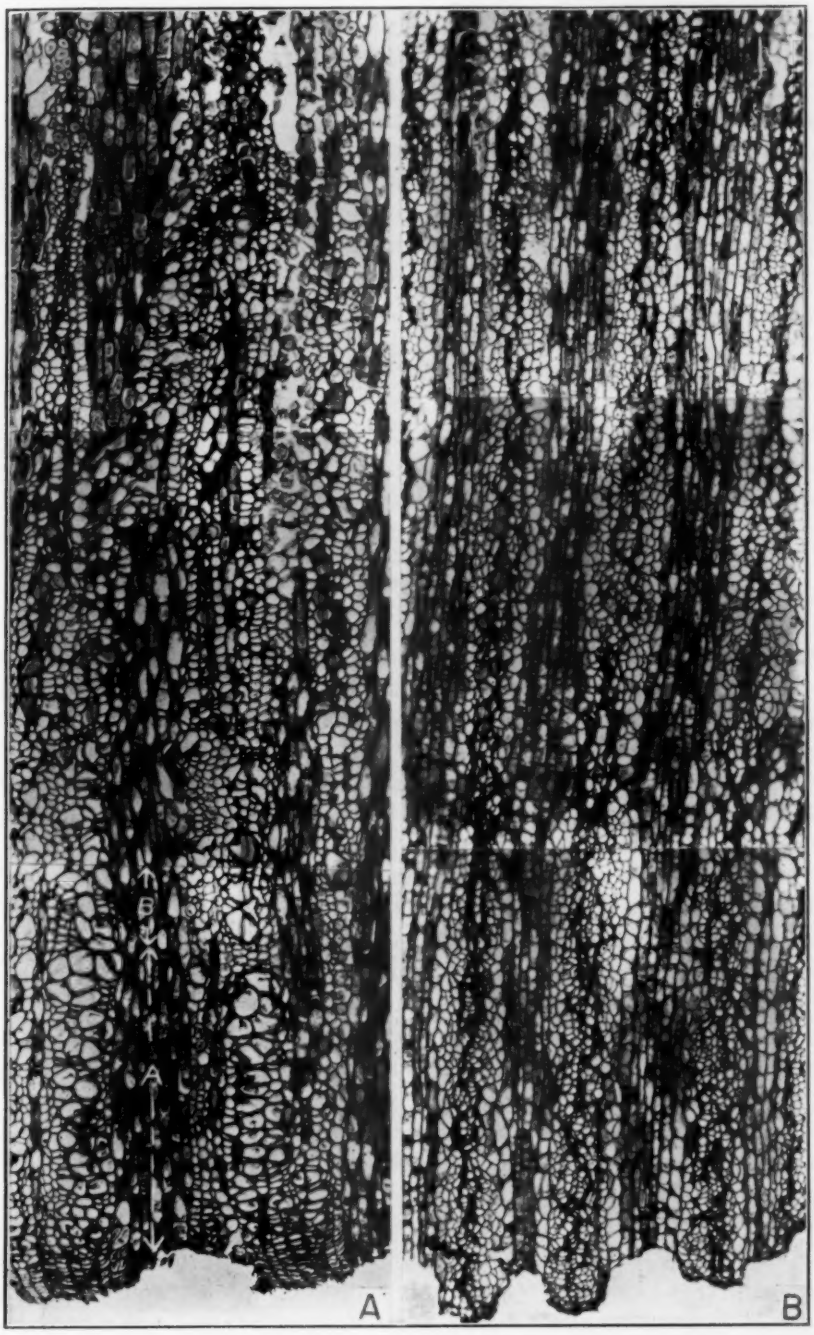

FIG. 10. Transverse sections from a buckskin-diseaesd Napoleon cherry tree on Mahaleb rootstock taken above and below the bud union. The tree was nine years old and was budded near the ground. It was inoculated in February, 1940, and the leaves were wilting and turning yellow in September, 1941, when the sections were made. A. Napoleon top. The width of phloem (A) between the arrows (320 μ) is composed of functioning phloem. The width (B) (180 μ) contains callused sieve tubes and the remainder of the phloem contains callused sieve tubes with wound gum. B. Mahaleb stock. The sieve tubes are collapsed to the cambium. (Both × 110.)

Two trees which survived the first year they were inoculated and which were examined the following spring after a few large sieve tubes had been produced had no wound gum or callus in the winter or new spring phloem; but a strong reaction occurred in the previous summer's phloem.

There was a similar distribution of wound gum in buckskin-diseased Early Richmond cherries (*Prunus cerasus* Linn.) and in *P. molis* (Walp.) when on Mahaleb. The injury was most extensive below the graft unions.

Histochemical studies have shown that the wound gum in the sieve tubes of diseased cherry has the same properties as that in diseased peach.

These observations perhaps indicate that the virus is elaborated in the Napoleon cherry tops and is then carried to the Mahaleb stocks by way of the phloem where severe necrosis results. The injury in the older phloem of the Napoleon tops is probably a secondary effect caused by necrosis of sieve tubes in the Mahaleb phloem. The injury was only apparent in the Mahaleb cherry phloem near the graft unions of trees in early stages of the disease, but it extended further down the trunk and roots in later stages.

Wound Gum in the Sieve Tubes of Diseased and Healthy Cherry Trees on Mazzard Roots

With the exception of one sample taken just beneath a canker in the cortex, buckskin-diseased and nonbuckskin cherry trees grown in orchards on Mazzard roots contained no wound gum. No wound gum was in branches from three trees suffering from zinc deficiency, in a branch of a tree suffering from bacterial gummosis, and in eight sections from branches and roots of five buckskin-infected trees. The absence of wound gum and phloem degeneration is in keeping with the relatively mild external symptoms in buckskin-diseased trees grown on Mazzard stocks.

Young Napoleon cherry trees on Mazzard roots which were grown in the greenhouse and were infected with the Green Valley and Palo Alto strains of virus had some wound gum in the phloem. In the Mazzard rootstock of one normal-looking tree which had been inoculated with Green Valley buckskin virus three years earlier, there were several bands of wound gum in the nonfunctioning phloem of previous years in the trunk, tap root, and branch roots. The nearest sieve tubes which contained wound gum were 400 microns from the cambium immediately below the bud union and 300 microns from the cambium at a point five inches below the bud union. The current season's phloem was apparently not affected. The Napoleon top at three levels had only occasional sieve tubes with wound gum, and they were 600 microns from the cambium at a point just above the union. Another normal-looking tree infected by the same virus gave a mild reaction in the outer margin of the summer phloem in a section a few inches from where a branch was inarched to a diseased sour cherry tree (*Prunus cerasus*). Above the bud union a reaction of medium intensity was obtained 400 microns from the cambium. The color was less intense below the bud union but occurred only 230 microns from the cambium. Six inches below the union in the tap root

the coloration was spotted, and it was absent in a lateral root. A normal-looking cherry tree on Mazzard root stock inarched to a peach tree infected with the Palo Alto virus showed only spotted reactions in five sections taken at points throughout the tree. The affected sieve tubes were 300 to 500 microns from the cambium. Another Napoleon tree carrying peach scions affected by the Palo Alto strain of virus on several top branches showed mild injury in the branches below the scions, medium injury in the trunk just below the branches, mild reactions above and below the bud union, and none at all in the roots. The reactions were observed 200 to 270 microns from the cambium in the outer summer phloem. Two of three sour cherries on Mazzard roots gave light reactions in stems of young greenhouse trees but little or no gum was present above and below the bud unions.

The results indicate that the diseased young sweet cherry trees on Mazzard stocks grown in the greenhouse were more susceptible to sieve-tube injury than large orchard trees. Possibly because of genetic variability, some Mazzard seedling root stocks showed more phloem injury than others. Also, several peach scions infected with Palo Alto virus caused more injury in the cherry being inoculated than was obtained with single cherry scions infected with Green Valley virus, or than was obtained by inarching the ends of branches of diseased trees to the trees being inoculated. The amount of injury observed in trees on Mazzard stock did not in any case approach that in sweet cherries on Mahaleb stock.

Two noninoculated Mazzard seedlings about three feet tall which were growing in five-gallon cans contained no wound gum in the sieve tubes although one of them was dying (apparently from bacterial gummosis). A healthy Napoleon tree on Mazzard roots showed no injury above and below the union, but occasional sieve tubes in the tap root contained wound gum.

Anatomy of Swollen Veins of Diseased Cherry

Leaves with swollen veins were collected from a buckskin-diseased Napoleon cherry tree on Mazzard roots in Green Valley, California, in August, 1941. Paraffin sections of a lateral vein showed that the swelling was caused by excessive amounts of new secondary xylem and phloem. The sieve tubes had very thick nacré walls and the metaphloem sieve tubes were partially crushed. About half of the cells between the phloem rays were sieve tubes and about half were parenchyma cells. Sieve plates of the sieve tubes in the older half of the phloem were callused.

Leaves from the healthy trees did not have the wide band of secondary tissue in the lateral veins, and the nacré walls of the metaphloem sieve tubes were very thick and almost filled the lumina of the cells. No definitive callus was present. Unlike the lateral veins of the peach, those of the cherry contained protophloem fibers.

DISCUSSION

The effect of the buckskin virus on the sieve tubes of the secondary phloem of peach trees and of sweet cherry trees when on Mahaleb rootstock

resembles that of the potato-leaf-roll virus on the primary sieve tubes of potato plants. The histology of plants infected by leaf-roll virus has been studied extensively. Esmarch (6) and Esau (5) reviewed the literature on the subject. As Esau has pointed out some of the workers have studied plants with a mixture of virus diseases and therefore their results are difficult of interpretation. Judging by the studies (1, 2, 13, 19) in which the authors have taken precautions to use plants with only the leaf-roll virus, the following changes occur in the sieve tubes and companion cells. The walls thicken and a separation between the primary walls occurs. Yellowing of walls and contents takes place and a red color is obtained when the sections are treated with phloroglucinol and hydrochloric acid. The sieve tubes are crushed by the turgor of adjacent parenchyma cells. Necrosis is confined to sieve tubes and companion cells and occurs in the older parts of the phloem.

Necrosis of sieve tubes of the secondary phloem in peach and in sweet cherry on Mahaleb stock resulting from the buckskin disease resembles the necrosis of the sieve tubes in the primary phloem in potato plants with leaf roll, except that no swelling of walls or separation of primary walls has been observed. In fact, the walls become thinner (Fig. 2).

Many virus diseases affecting the phloem affect parenchyma cells near the sieve tubes. Esau (3, 4) reports that in leaves of sugar beets affected by the curly-top disease parenchyma cells adjacent to the sieve tubes first become hypertrophied and parenchyma cells further removed are subject to hyperplasia. The hyperplastic cells develop into abnormal sieve tubes. These abnormal tissues may then become necrotic, and adjacent parenchyma cells undergo proliferation and division. Magee (12) in his studies on the bunchy top of banana also found that parenchyma cells adjacent to sieve tubes underwent hypertrophy and hyperplasia. Parenchyma cells are usually not affected in buckskin-diseased plants; although, in severe cases necrosis of the entire phloem and cortex may occur and a hyperplastic condition may arise as a result of abnormal activity of the cambium in leaves. Fiber initials may also become necrotic in young tissues.

A recently described virus disease, phloem necrosis, of tea may resemble buckskin in peach. Some cells of the phloem are said to be collapsed and yellow, whereas adjacent cells appear to be normal in the photomicrograph shown (7). Necrosis also occurs in the midribs of diseased tea leaves and from the photomicrograph it appears as though new abnormal secondary phloem is produced in a manner similar to that found in buckskin-diseased peach. In a later paper it was reported (8) that severe vein-swelling occurs. Necrosis is said to be visible macroscopically in the phloem area in more advanced stages when tangential slices are removed from large roots.

When sweet cherry trees on Mahaleb roots are infected, extensive sieve-tube necrosis results in the Mahaleb stocks but only a small amount of necrosis occurs in the sweet cherry tops. The injury in the Mahaleb is immediately below the bud union when symptoms first appear, but in advanced

stages in orchard trees it has been found a foot or more below the union. The tree eventually dies as a result of the disease. Although Mahaleb rootstocks have been considered virtually immune to buckskin virus and have been extensively used for this reason in orchards affected by the Green Valley strain of virus, it seems probable now that Mahaleb trees are really hypersensitive, suffering such extensive injury to the phloem that movement of the virus is inhibited. The distal portions of the Mahaleb roots of affected small trees with Napoleon tops are often killed before the top is entirely dead. However, this may be an indirect effect of the drastic reduction in functioning phloem between top and roots, chiefly in the upper portion of the Mahaleb stock.

Sweet cherries are not completely compatible with Mahaleb root stock (10) as is evidenced by their small size and shorter life as compared to trees on Mazzard stock. Sections above and below the bud union indicate that the area of phloem is larger in the sweet cherry than in Mahaleb cherry. Sieve tubes of the Mahaleb stocks form definitive callus earlier in the autumn than do the sieve tubes of the sweet cherry tops. These differences in available phloem for translocation may be the cause of necrosis observed in the sieve tubes of healthy trees and the seeming incompatibility in certain situations. It should be emphasized, however, that when trees are infected with buckskin virus the injury becomes much more pronounced and causes severe necrosis during the summer as well as in the autumn; and ultimately all of the Mahaleb sieve tubes are killed. In healthy trees most of the necrosis occurs in the Napoleon tops.

Callusing of sieve plates followed by subsequent collapse of sieve tubes can be caused by several agencies and treatments. They are ringing, grafting on incompatible rootstocks, environmental conditions leading to dormancy, introduction of dilute solutions of eosin (18), and presence of virus (buckskin virus in peach and cherry and potato-leaf-roll virus in potato). It seems probable that interference with translocation or the presence of a toxic substance may be the cause of sieve-tube necrosis, especially in case of virus infections. Presence of virus in one part of the phloem could cause necrosis, upset normal translocation, and lead indirectly to sieve-tube necrosis in other parts of the plant. Possibly this happens when diseased Napoleon cherries are on Mahaleb roots. Normally the sweet cherry sieve tubes are little affected by buckskin virus when on Mazzard roots, but when the sieve tubes of Mahaleb stock become necrotic the subsequent necrosis of sweet cherry sieve tubes may, at least in part, be a secondary effect due to the death of the Mahaleb sieve tubes. Products of metabolism rather than the virus itself may be the immediate cause of some of the symptoms seen in infected plants.

A positive test with phloroglucinol and hydrochloric acid for wound gum in sieve tubes may be of value as a diagnostic symptom for buckskin disease in peach. It would be of value, however, only if stems of large diameter were taken at some distance from injuries and from trees grown on a com-

patible rootstock. Branches showing leaf symptoms are most apt to give a positive test. A positive test should be considered only as another symptom of buckskin disease and not specific proof of it without accessory evidence.

SUMMARY

Necrosis of sieve tubes occurs in buckskin-diseased peach trees. Necrotic sieve tubes contain a substance which is here called wound gum. This substance gave a positive test with phloroglucinol and hydrochloric acid and a negative, or a weakly positive, reaction with the Maule reagents for lignin. It was insoluble in a mixture of potassium chlorate and hydrochloric acid, and in hot 10 per cent nitric acid. After the wound gum was removed by alternate treatments with chlorine water and sodium sulphite, the sieve tubes gave a positive test for cellulose although they did not do so before the wound gum was removed. Usually necrosis was limited to the sieve tubes, but sometimes it also involved other cells.

In young peach shoots with early stages of necrotic spotting in the leaves, necrosis of sieve tubes was confined to leaves with symptoms and to the leaf traces below the affected leaves. As the virus spread into the stem tips, sieve tubes of the youngest leaf primordia which had developed these elements became necrotic. The oldest sieve tubes became necrotic first. If, after the onset of disease, growth was resumed, new sieve tubes usually failed to undergo turgor expansion, lacked nacré walls, and early became necrotic.

When certain strains of the buckskin virus or very susceptible peach varieties were used, cankers developed on the stems. The cankered areas contained gum pockets in the cortex and phloem. Such areas were isolated by periderms. Gum pockets in some instances appeared in the unlignified new xylem.

Swollen veins in infected peach were characterized by abnormal production of phloem. Sieve tubes of this abnormal phloem soon became necrotic, as did also those of the original phloem.

Ringing of the stems caused swollen veins to develop in peach. Sieve tubes in the stems immediately above the rings collapsed and small amounts of wound gum sometimes developed in them. New phloem was formed in which the sieve tubes failed to undergo turgor expansion. Gum pockets formed in newly initiated unlignified xylem.

Peach scions on Myrobalan root stock often grow normally at first, but later exhibit yellow, rolled leaves with swollen veins and some premature defoliation. Such stems contained necrotic sieve tubes with wound gum. New phloem was initiated in which sieve tubes did not undergo turgor expansion.

In uninfected young sweet cherry trees on Mahaleb stocks grown in pots, occasional sieve tubes on the outer margin of the current year's summer phloem and the non-functioning phloem of previous seasons in some cases contained wound gum. In the summer phloem of healthy orchard trees wound-gum formation occurred frequently in the autumn just before trees

became dormant. In autumn the Mahaleb sieve tubes were observed to form callus before the sweet cherry sieve tubes. Wound gum was more abundant in the sweet cherry tops than in the Mahaleb stock. The reverse was true in buckskin-infected trees.

In infected cherry trees on Mahaleb stock, wound gum formation was very extensive even in midsummer in the summer phloem. Ultimately the youngest sieve tubes were affected in the Mahaleb stock just below the bud unions.

No injury to phloem has been observed in either diseased or healthy orchard trees on Mazzard roots. Some wound gum was present in the outer phloem in infected young greenhouse trees on this stock.

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A ROOT ROT OF GUAYULE CAUSED BY PYTHIUM ULTIMUM

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INTRODUCTION

A root rot of guayule (*Parthenium argentatum* Gray) nursery seedlings from which a *Pythium* was consistently isolated was reported[1] as prevalent in the nurseries at Salinas, California, in 1942. The following year there was a re-occurrence of the disease and it was also reported from the nurseries near Oceanside,[2] California. Its prevalence in the nurseries at Salinas in 1944 was limited because of the small acreage planted. In both 1943 and 1944 the disease was found in direct field seedlings in the Salinas Valley.

The causal fungus, identified as *Pythium ultimum* Trow, attacks the roots of young guayule seedlings over a considerable time and causes characteristic symptoms. When the roots of plants in the cotyledon stage are affected, the disease is termed "seedling root rot." If the tap roots or root crowns of older seedlings are attacked and the woody cylinder at the lesion is pinkish or reddish, the disease is called "pink rot." The loss from seedling root rot was usually greater than the loss from the pink rot stage of the disease which occurred on older plants.

SEEDLING ROOT ROT

Seedling root rot of guayule may develop shortly after emergence and continue to affect the plants in the cotyledon stage or during a period of approximately four weeks. The first observed symptom is a slight stunting associated with a purplish or reddish color of the cotyledons. The roots of affected plants are rotted at varying depths in the soil and the degree of stunting depends upon the extent of injury to the root systems (Fig. 1, A). Lesions that develop on the tap root near the soil surface usually result in severe stunting or the death of the seedlings. Infections which develop deeper in the soil and which are limited to a few lateral roots may not greatly affect the growth rate or appearance of the plants. The fungus may invade and destroy most of the root system or be confined to a small area at the point of infection. Because new lateral roots develop above the lesion on the tap root, a plant affected by seedling root rot usually has a forked or multiple tap root.

Seedling root rot of guayule may be regarded as a part of the damping-off disease complex with symptoms sufficiently distinct to separate it from post-emergence damping-off. Infected seedlings, even though much of their root systems may be destroyed, usually survive and under good growing conditions tend to overcome the initial loss of roots. The recovery of diseased seedlings from fungus attack is associated with a reduction in

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soil moisture in the upper 2 to 3 inches of soil and an increase in the age of the seedlings. Ordinarily, satisfactory emergence is obtained in 10 to 14

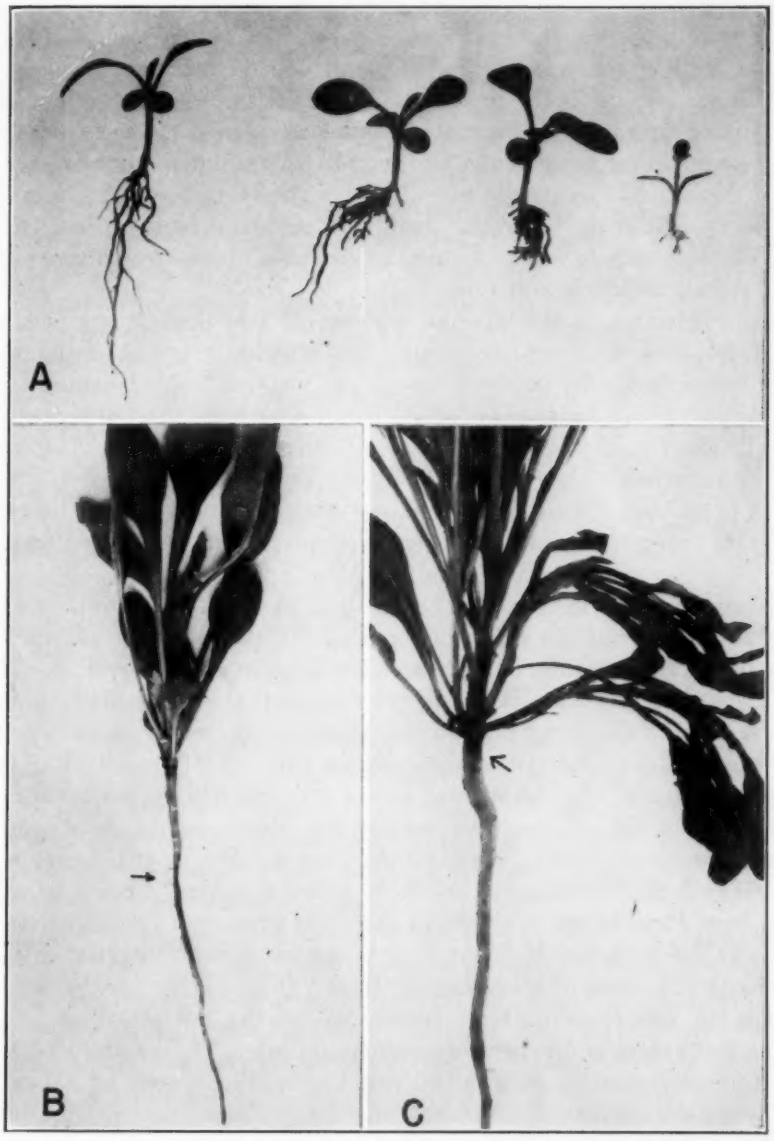

FIG. 1. Pythium root rot of guayule. A. Seedling root rot on very young plants. New root development has been initiated above the diseased parts in all four seedlings. (Approximately natural size.) B and C. The pink rot stage of Pythium root rot. The arrows point to lesions on the tap root; (B) at some distance below the soil surface; and (C) at the root crown. (Approximately one-half natural size.)

days after sowing, after which the frequency of irrigation is reduced and the soil moisture conditions become less favorable for fungus infection near the soil surface.

PINK ROT

The pink rot stage of Pythium root rot of guayule seedlings ordinarily develops on plants 6 to 16 weeks old. The first evident symptom of the disease is wilting during the heat of the day. The severity of wilting and eventual death or recovery of affected plants depends upon the location and extent of the lesions on the tap roots. Plants with circumferential lesions on the upper tap root or at the root crown usually do not recover; those with lesions on the lower tap root deep in the soil wilt slightly during the warm part of the day but recover in a few days. The small feeder roots may be attacked as well as the tap root. Injury to the small roots ordinarily does not cause the plants to wilt because of the number of uninfected roots available to supply moisture to the top.

Pink rot lesions on the tap root are rarely more than $\frac{1}{8}$ to $\frac{1}{2}$ inch long (Fig. 1, B), and occur most frequently 3 to 6 inches below the soil surface. In the early stages the infected tissues are watersoaked, translucent, and somewhat pinkish. Later the diseased bark becomes soft and depressed and it usually sloughs off when the plant is pulled from the ground. The woody cylinder underneath the lesion and for a short distance above and below is pinkish or reddish. This characteristic color serves as a diagnostic aid in separating root rot caused by *Pythium ultimum* from that caused by other fungi.

Infections which develop at or slightly above the root crown are commonly associated with the piling of earth against the lower leaves and stems of the plants by cultivators or by machines used to apply fertilizer. After such operations, provided the beds are irrigated, there is usually a sharp increase in the number of pink-rotted plants which reaches a peak within 3 or 4 days. Crown infections were induced experimentally by piling earth against the plants. The number of plants infected was proportional to the depth to which the soil was piled against the plants and to the amount of irrigation water applied. There is a tendency for lesions at the root crown to increase during prolonged periods of foggy weather. Crown lesions in plants from 12 to 16 weeks old often cause the progressive death of the top branches; and such plants in the early stages of the disease may be recognized by the presence of conspicuous "flags" (Fig. 1, C).

Pink rot was observed most frequently on the heavier types of soil. Instances of severe and extensive losses from pink rot followed 4 to 6 hours of continuous irrigation with the overhead sprinkling system on heavy soil. Also, losses were severe on low areas where water accumulated from irrigations of shorter duration. Losses were relatively unimportant on the lighter and well-drained soils.

THE CAUSAL FUNGUS

The association of *Pythium sp.* with seedling root rot and pink rot of guayule was established in the course of routine isolations from diseased plants. The pathogenicity of the fungus was demonstrated by pure culture

inoculation tests made in the greenhouse, and its identity as *Pythium ultimum* Trow confirmed by comparison[3] and examination of 51 isolates.

The macroscopic appearance and growth rates of the 51 isolates were similar in culture. However, only 27 isolates produced oogonia and antheridia on corn-meal agar. The remaining 24 isolates produced only sporangia or sporangia-like structures. All attempts to obtain oogonia and antheridia by growing these isolates on the culture media usually used for this purpose were unsuccessful.

The 27 isolates that produced oospores in culture agreed in all essential respects with an isolate from guayule identified by John T. Middleton as *Pythium ultimum* Trow. Although many of the isolates failed to produce oospores in culture, they were considered as referable to *P. ultimum* because of their similar morphological appearance and comparable rate of growth. This conclusion was strengthened by the common source of the isolates in that they were obtained from the same diseased material. This viewpoint is in agreement with Tompkins, Ark, Tucker, and Middleton[4] who reported the isolation of a strain of *P. ultimum* from Zucchini pumpkin fruits which did not produce oospores. Furthermore, there was a progressive gradation in the isolates from those that produced an abundance of oospores and few sporangia to those that produced only sporangia or sporangia-like structures. This behavior in respect to spore formation is suggestive of the dual phenomenon of Hansen[5] which is common in imperfect fungi but which has not been demonstrated in the Phycomycetes.

SUMMARY

Pythium ultimum Trow was determined as the causal agent of a root rot of guayule (*Parthenium argentatum* Gray) nursery seedlings in California. Two stages of the disease are described; seedling root rot which affected plants in the cotyledon stage and "pink rot" which affected plants from 6 to 16 weeks old. The over-all losses in the nurseries were not serious, however, the disease occasionally caused considerable mortality in localized areas especially on heavy poorly drained soil.

Twenty-seven of the 51 isolates of the fungus produced oospores in culture and were considered typical *Pythium ultimum*. Twenty-four of the isolates produced only sporangia or sporangia-like structures in culture but were referred to *P. ultimum* on basis of association with oospore-producing isolates, morphological appearance, and growth rates.

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A SIMPLE METHOD OF INOCULATING BARLEY WITH LOOSE SMUT[1]

J. M. POEHLMAN

(Accepted for publication March 26, 1945)

Winter barley varieties commonly grown in Missouri are highly susceptible to loose smut, *Ustilago nuda* (Jens.) Rostr. The breeding of winter types resistant to this disease has been handicapped by the lack of information regarding the relative resistance of common varieties and the scarcity of parent material with high resistance. Much of this lack of information results from difficulties encountered in the artificial inoculation of breeding material. In the winter of 1942–43, 708 varieties of barley, representing the winter types in the U. S. Department of Agriculture barley C.I. collection, were grown at Columbia, Missouri. Many of these varieties were introductions from foreign lands and little or nothing was known regarding their reaction to loose smut. The possibility that some winter types with good resistance to loose smut could be found, led the author to carefully survey the material at hand.

The partial vacuum technique for inoculating barley with loose smut as described by Moore (3) and used by other workers, (1, 2, and 4), had been used by the author in the previous season with fair success. Three heads from each of 40 varieties and selections of winter barley had been inoculated. The percentage of smutted heads from the inoculated seed (average of 40 varieties) was 22.6 per cent as compared to 0.6 per cent in uninoculated checks. But several objections to this method were evident. The equipment was clumsy and the procedure time-consuming when employed in the field. The early flowering of barley, sometimes even before emergence from the boot, made it necessary to inoculate while the culms were very tender. Injury to the culms or even breakage frequently occurred. Failure of the glumes to open properly when the vacuum was applied sometimes resulted in poor inoculation. Because inexperienced help was used for this work, a simple, positive and efficient method of inoculation, that could be quickly learned, was necessary if many varieties were to be tested.

Some preliminary inoculations made in preceding years in the field and in the greenhouse suggested a simple procedure that could be successfully used. The method and the results from its use are described.

DESCRIPTION OF METHOD

The equipment used consisted of a hypodermic needle (1 inch, 25 gauge) inserted into a rubber bulb (10 cc. capacity). The bulb was filled with a suspension of chlamydospores of *Ustilago nuda*. A few drops of this suspension was injected into each floret by piercing the lemma with the needle and squeezing the bulb slightly. Inoculations were made one to two days after

[1] Missouri College of Agriculture Journal Series No. 969.

the spike broke the boot. At this stage the majority of the florets had reached or just passed pollination. Immature florets at the base of the spike were removed. If the variety was awned, the awns were usually clipped to facilitate inoculation but this was not necessary. After inoculation each spike was tagged for identification. Spikes inoculated in this manner developed normally with no apparent injury and with little if any reduction in seed setting except from the removal of the immature spikelets.

The suspension of chlamydospores was prepared in the field from fresh inoculum. Adjacent field plots in 1943 contained ten varieties of winter barley, all of which were infected with loose smut (*Ustilago nuda*) in varying amounts. These barleys had been grown from commercial lots of seed obtained from several locations in Missouri, Oklahoma, Indiana, or Ohio. This offered an opportunity to use inoculum of widely scattered origin. Each day smutted heads were selected from all of these varieties, chopped into small pieces, placed in a small square of cheese cloth, and immersed in tap water. The smut spores were strained through the cheese cloth similar to the manner in which a tea bag is used. By this method a concentrated spore suspension could be prepared without inclusion of extraneous plant materials that might clog the hypodermic needle. Sufficient dextrose was added to the suspension to make a one per cent solution and the contents were shaken vigorously.

The rubber bulbs held sufficient inoculum to inoculate 15–20 separate spikes. As the bulbs fill slowly, an extra bulb was carried in a beaker of suspension by each worker to avoid loss of time. Varieties and selections to be inoculated were listed on index cards to which were attached envelopes containing a labeled tag for each head to be inoculated. Tags of a specific color were used for labeling of heads inoculated with smut so that they could readily be identified. After some skill had been attained, 30 to 40 heads could be inoculated per hour by a worker.

Three heads were inoculated from each variety or selection. These three heads, along with two uninoculated heads, were harvested, each head threshed separately and the seed planted in a one-foot row in the field in the fall of 1943. Removal of the immature spikelets reduced the number of seeds from each head but by planting in a one-foot row a dense stand could be obtained. Previous experience had demonstrated that thinly spaced plants on the soil type present at Columbia, Missouri, frequently heave badly and would thus be lost. This rate of planting precluded taking notes on individual plants so counts of normal and smutted heads were made in each row the following spring. Percentages of smutted heads in each variety were calculated as the average from the three rows.

MATERIAL INOCULATED AND RESULTS

Three groups of material were inoculated as follows:

(1) 180 selections from the C.I. winter barley group (representing all selections from the 708 varieties that survived the winter at Columbia, Missouri, with a stand of 60 per cent or better).

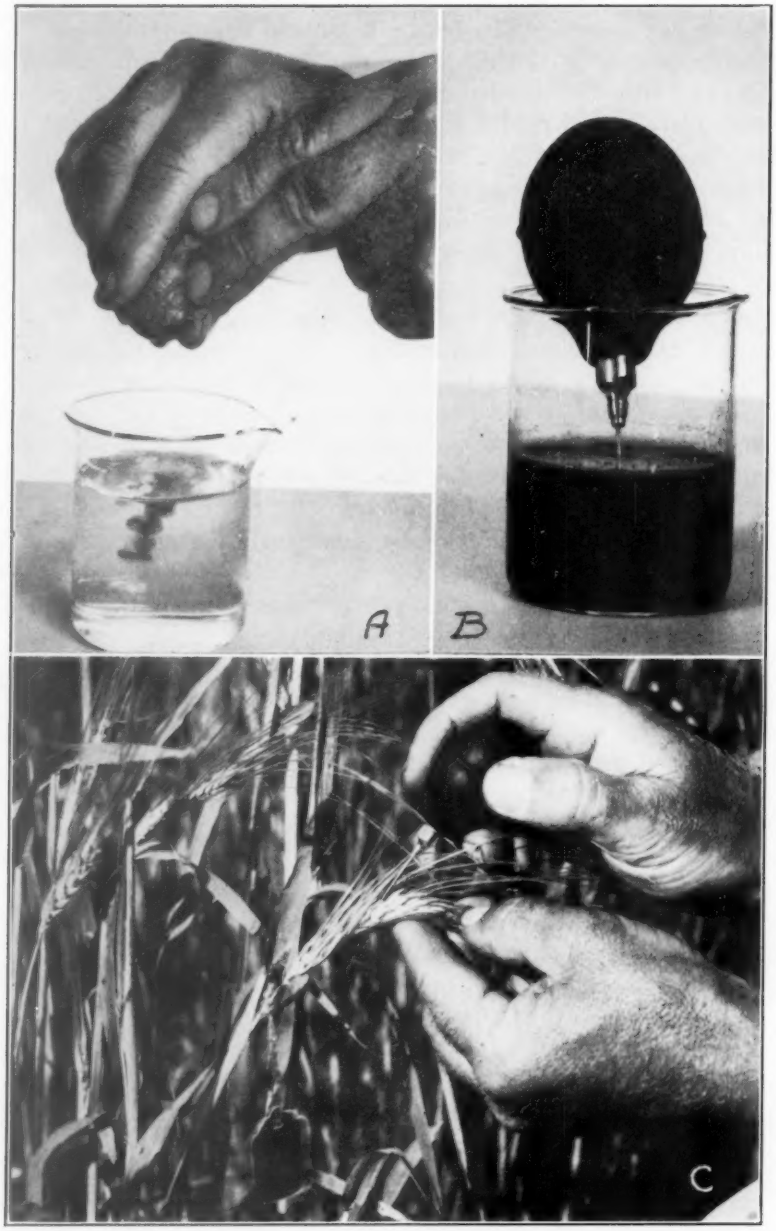

FIG. 1. Steps in the inoculation of barley with loose smut. A. Preparation of inoculum by straining smut chlamydospores through cloth and making a spore suspension in tap water plus dextrose. B. The rubber bulb fitted with hypodermic needle and ready for filling with inoculum. C. Inoculation of the barley florets.

(2) 42 standard varieties from nursery and uniform winterhardiness tests.

(3) 20 selections from the variety Missouri Early Beardless. Average percentages of infection, as determined by counts of smutted heads, in inoculated and uninoculated check rows of each group are listed here: (1) C.I. group, inoculated, 23.2 per cent, uninoculated checks, 1.5 per cent; (2) standard variety group, inoculated, 22.1 per cent, uninoculated checks, 3.6 per cent; and (3) Early Beardless selections, inoculated, 10.2 per cent, uninoculated checks, 2.4 per cent. The distribution of the barley selections from these groups in infection percentage classes is summarized in table 1.

The percentages of smutted heads in the inoculated material as compared to percentages in the uninoculated checks demonstrate the effectiveness of the method. In the C.I. barley group of 180 selections (Table 1), 80 selec-

TABLE 1.—*Distribution of the barley selections in infection percentage classes*

Material	Infection percentage classes				
	0	0.1–20	20.1–40	40.1–60	60.1–100
C.I. barley group:					
No. of selections	42	58	38	27	15
Per cent of total	23.3	32.2	21.1	15.0	8.3
Standard variety group:					
No. of selections	8	14	12	7	1
Per cent of total	19.0	33.3	28.6	16.7	2.4
Early Beardless selections:					
No. of selections	8	7	4	1	.....
Per cent of total	40.0	35.0	20.0	5.0	.....

tions (44.4 per cent) contained over 20 per cent smutted heads, 42 (23.3 per cent) contained over 40 per cent smutted heads, and 15 (8.3 per cent) contained over 60 per cent smutted heads. A similar distribution is noted in the standard variety group. Resistance had previously been recorded in the Early Beardless selections, so lower infection percentages were expected in this group.

The number of selections in the C.I. group without smut (23.3 per cent) appears proportionally large. Of the 42 selections without smut, 11 had plants surviving from only two of the inoculated rows, while 13 had plants surviving from only a single inoculated row. All of these 24 selections were low in vigor and in each winter killing had been severe. That plants infected with loose smut are more susceptible to winter injury is suggested by winter killing notes on the inoculated and uninoculated rows. Survival in the inoculated rows was eight to ten per cent less than in the uninoculated checks. Similar results have been reported by other investigators (4, 5). In the 24 weak selections, infected plants may have been lost from winter injury. These may therefore represent escapes rather than resistant types.

Of the eight standard varieties reported without infection (Table 1), all are known to possess some resistance. In four of these, loose smut has not been observed previously in the nursery at Columbia, either from natural

or artificial inoculation. Two of the selections have previously contained small amounts of smut from natural infection. The other two varieties had not been grown at Columbia before. In three of the eight Early Beardless selections without infection, loose smut has never been observed either from natural or artificial inoculations, while in the other five slight infections from artificial inoculations or traces from natural infections have previously been observed.

All selections containing five per cent or less infection were reinoculated. Detailed reactions will be reported when these selections have been more fully checked.

DISCUSSION AND SUMMARY

A simple method of inoculating barley with loose smut (*Ustilago nuda*) is described. Infections obtained by this method in a wide range of barley varieties demonstrate that it may be successfully used to sort out susceptible and resistant varieties. Percentage infections in individual varieties inoculated by this method are in the range previously obtained by the partial vacuum technique. The simplicity of the method described makes it adapted for field inoculation of large numbers of varieties or selections in a breeding nursery. It should prove useful for the plant breeder in finding barley varieties resistant to loose smut or in selecting resistant lines from segregating material.

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SMUT CONTROL IN SORGHUM AND EFFECT OF DUST FUNGICIDES AND STORAGE ON EMERGENCE[1]

R. W. LEUKEL AND J. E. LIVINGSTON[2]

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Excellent control of covered kernel smut (*Sphacelotheca sorghi* (Lk.) Clint.) in Sharon kafir was obtained in field experiments at 7 stations in 1942[3] by the use of several dust fungicides at the generally recommended dosages, and at $\frac{1}{2}$ and $\frac{1}{4}$ of these dosages. The relatively low average percentage of smutted heads (25.3 per cent) from untreated seed rendered those results somewhat inconclusive and made it desirable to obtain more data on the control of kernel smut by some of these fungicides and their effect on emergence, especially when treated seed is stored.

Similar tests, therefore, were carried out at 5 stations in 1943 and at 2 stations in 1944. Several of the fungicides used in 1942 were replaced in 1943 by others, some of which proved unsatisfactory and were replaced by still others in the 1944 tests.

MATERIALS AND METHODS

The following fungicides were used:

1. N.I. (New Improved) Ceresan (5 per cent ethyl mercury phosphate).
2. Copper carbonate, containing 50 per cent metallic copper.
3. Spergon (98 per cent tetrachloro parabenzoquinone).
4. Arasan (50 per cent tetramethylthiuramdisulfide).
5. DuBay 1452–C (6.5 per cent ethyl mercury p-toluene sulfonanilide).
6. Leytosan (7.2 per cent phenyl mercury urea).
7. Merc-o-dust, a compound of uncertain composition purported to contain 1.5 per cent mercury and 2 per cent formaldehyde.
8. M.T.D.S. (morpholine thiuramdisulfide).
9. Fermate (70 per cent ferric dimethyl dithiocarbamate).
10. U.S.R. 604 (dichloro naphthoquinone).
11. Zimate (zinc dimethyl dithiocarbamate).
12. Basic copper sulfate, containing 50 per cent metallic copper.
13. Sulfur (300-mesh dusting sulfur).

Numbers 1, 2, 3, 4, and 13 were used both years. Numbers 5, 6, 7, and 8 were used in 1943, and 9, 10, 11, and 12 in 1944 only. Seed of Leoti sorgo and Sharon kafir was used both years, the first because it is susceptible to seed injury by certain fungicides, and the second because it is highly sus-

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Nebraska Agricultural Experiment Station. Journal Series Paper No. 368 of the Nebraska Agricultural Experiment Station.

[2] Pathologist, Division of Cereal Crops and Diseases and Assistant Pathologist, Nebraska Agricultural Experiment Station, respectively.

[3] Leukel, R. W. New fungicides and reduced fungicide dosages for the control of kernel smut of sorghum. Phytopath. 32: 1091–1093. 1942.

ceptible to covered kernel smut and has an upright, low habit of growth which greatly facilitates taking data on smut control. The seed after being thoroughly cleaned was dusted with viable spores of covered kernel smut at a 1 to 100 spore dosage (by weight) after which separate portions were treated with the different fungicides to be tested, and planted in field plots in triplicated 44-foot rows at the rate of 200 seeds per row. Stand counts were made after the plants had had ample time to emerge. Data on smut control were taken by counting the total number of heads and the smutted heads in each row.

TABLE 1.—*Effect of seed treatment with certain dust fungicides on emergence in two varieties of sorghum planted at the rate of 200 seeds per 44-foot row at several stations in 1943*

Seed treatment		Emergence in					
Material	Rate per bushel	Sharon kafir[a]			Leoti sorgo[b]		
		Aver.[c]	Difference	Odds	Aver.[d]	Difference	Odds
	Oz.	*Pct.*			*Pct.*		
New Improved Ceresan	½	68.8	20.8 ± 4.5	1: 9999	39.4	− 9.9 ± 1.7	*
do	¼	65.9	17.9 ± 2.9	*	49.6	0.3 ± 3.9	1: 1
Copper carbonate	2	69.2	21.2 ± 2.3	*	55.1	5.8 ± 2.6	1: 35
do	1	64.9	16.9 ± 2.7	*	48.8	− 0.5 ± 1.3	1: 2
Spergon	2	62.4	14.4 ± 3.4	1: 2998	47.3	− 2.1 ± 3.8	1: 2
do	1	59.5	11.5 ± 3.2	1: 627	48.5	− 0.8 ± 2.4	1: 2
Arasan	2	65.3	17.3 ± 2.3	*	57.7	8.4 ± 2.8	1: 133
do	1	62.4	14.4 ± 3.1	1: 9999	55.9	6.6 ± 2.4	1: 62
DuBay 1452-C	½	67.7	19.7 ± 2.6	*	34.8	−14.5 ± 1.9	*
do	¼	66.3	18.3 ± 2.5	*	57.8	8.4 ± 2.7	1: 169
Leytosan	½	59.2	11.2 ± 1.8	*	52.6	3.3 ± 3.7	1: 4
do	¼	59.3	11.3 ± 1.9	*	49.3	0.0 ± 3.3	1: 1
Morpholine thiuramdisulfide	2	57.5	9.5 ± 2.5	1: 1110	48.8	− 0.5 ± 2.9	1: 1
do	1	54.8	6.8 ± 2.2	1: 244	50.8	1.5 ± 2.8	1: 2
Mere-o-dust	½	49.0	1.0 ± 2.1	1: 2	43.7	− 5.7 ± 1.6	1: 293
do	¼	47.9	− 0.1 ± 1.8	1: 1	47.7	− 1.7 ± 0.5	1: 293
Sulfur	2	46.0	− 2.0 ± 2.0	1: 5	35.3	−14.0 ± 2.6	1: 4999
do	1	44.0	− 4.0 ± 2.2	1: 16	41.2	− 8.2 ± 3.5	1: 40
Check		48.0			49.3		

* Odds greater than 1: 9999.

[a] Three replications planted at each of 6 locations.

[b] " " " " " " 4 " "

[c] Each figure is based on 3600 seeds planted.

[d] " " " " " 2400 " "

EXPERIMENTAL RESULTS

In 1943, separate lots of smutted seed of Sharon kafir and Leoti sorgo were treated with 9 fungicides at two rates of application. Sharon kafir was planted at five stations[4] and Leoti at three stations. Two plantings were made at Lincoln, Nebraska, one on May 22 and another on June 17. The data on emergence and on the control of covered kernel smut are summarized in tables 1 and 2, respectively.

[4] The seed was planted and the data on emergence and smut control taken by the following: E. G. Heyne, Manhattan, Kansas; A. F. Swanson, Hays, Kansas; A. E. Lowe, Garden City, Kansas; and O. J. Webster, Lincoln, Nebraska. Their kind cooperation is gratefully appreciated.

The data on emergence were sufficiently similar at the different stations to be analyzed as a single experiment, using Student's method[5] to compare the results from each lot of treated seed with results from untreated seed. Emergence in Sharon kafir was significantly improved by 7 of the fungicides. Emergence in Leoti was significantly impaired by N.I. Ceresan, DuBay 1452–C, Merc-o-dust, and sulfur applied at the generally recommended rates of treatment, and significantly improved by Arasan applied at 2 ounces, and DuBay 1452–C at ¼ ounce per bushel.

TABLE 2.—*Control of covered kernel smut in Sharon kafir and Leoti sorgo, grown from seed that had been inoculated at a 1 to 100 spore dosage, treated, and planted at several stations*[a] *in 1943*

Seed treatment		Percentage smutted heads in											
Fungicide	Rate per bu.	Sharon kafir at						All stations	Leoti sorgo at				All stations
		G.C.	H.	B.	L–1	L–2	M.		B.	L–1	L–2	M.	
	Oz.												
None	....	33.8	3.3	24.3	37.3	27.9	14.1	23.5	10.0	19.5	6.1	13.0	12.2
N.I. Ceresan	½	0.2	0.0	0.0	0.0	0.7	0.0	0.2	0.0	0.0	0.0	0.0	0.0
do	¼	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Copper carbonate	2	0.0	2.1	0.0	0.0	0.0	0.0	0.4	0.4	1.2	0.0	1.6	0.8
do	1	0.0	1.1	0.0	0.0	0.0	0.0	0.2	0.4	1.3	2.4	0.6	1.2
Spergon	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
do	½	0.0	0.0	0.3	0.0	0.0	0.0	0.1	0.8	0.0	0.3	0.3	0.4
Arasan	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
do	½	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.2
None	....	36.4	3.7	22.9	32.9	24.5	6.0	21.1	14.1	25.0	6.6	10.5	14.1
DuBay 1452–C	½	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0	0.0	0.7
do	¼	0.4	1.1	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Leytosan	½	0.0	0.0	0.0	0.7	1.4	0.0	0.4	0.0	0.3	0.0	0.0	0.1
do	¼	0.8	0.0	0.0	0.0	0.7	0.0	0.3	1.7	5.2	1.2	2.1	2.6
M.T.D.S.	1	0.0	0.0	0.0	0.4	0.0	0.0	0.1	0.9	2.4	0.4	2.5	1.6
do	½	0.2	0.0	1.0	1.7	0.7	0.4	0.6	1.1	7.1	1.9	1.6	3.0
Merc-o-dust	½	35.3	3.6	30.9	41.7	24.2	7.0	23.8	16.3	14.2	4.0	9.0	11.0
do	¼	40.1	5.6	26.8	52.0	20.3	10.0	25.8	8.6	20.2	6.2	8.2	10.8
Sulfur	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.4	0.2
do	1	0.7	0.0	0.0	0.5	0.0	0.0	0.2	0.0	0.0	0.0	0.3	0.1

[a] Stations were: G.C.—Garden City, Kans.; H.—Hays, Kans.; B.—Beltsville, Md.; L–1 and L–2—Lincoln, Nebr. (2 plantings); and M.—Manhattan, Kans.

The percentage of heads infected with smut was again somewhat low for obtaining conclusive data on the fungicidal effectiveness of the treatments. In Sharon kafir the smut was controlled fairly well by all fungicides except Merc-o-dust, which proved worthless as a fungicide in both varieties. In Leoti an average of more than 1 per cent of the heads was smutted when grown from seed treated with copper carbonate (1 ounce), Leytosan (¼ ounce), and M.T.D.S. (1 ounce and ½ ounce).

Four of the fungicides used in 1943, namely, DuBay 1452–C, Leytosan, M.T.D.S., and Merc-o-dust, were replaced by Fermate, U.S.R. 604, Zimate, and basic copper sulfate in the 1944 experiments. These, along with the

[5] Student, Biometrika **6**: 1–25, 1908 and **11**: 414–417, 1917.

other five used in 1943, were applied at 2 rates to smutted seed of Sharon kafir and Leoti sorgo. Sharon kafir was planted at Beltsville, Maryland, and at Lincoln, Nebraska. Leoti sorgo was planted at Beltsville for studies on emergence. The data on emergence and smut control are presented in table 3. Arasan was somewhat superior to the other dusts with respect to its effect on emergence, although U.S.R. 604, on the whole, was almost equal to Arasan in this respect, and at Lincoln was superior to it. At Beltsville

TABLE 3.—*Effect of dust fungicides on emergence in sorghum and on control of covered kernel smut in 1944*

Seed treatment		Average stand[a] per row in					Smutted heads at Beltsville[c]
		Sharon kafir at			Leoti at Beltsville	Both varieties	
Fungicide	Rate per bu.	Lincoln	Beltsville	Lincoln and Beltsville[b]			
	Oz.	*No.*	*No.*	*No.*	*No.*	*Pct.*	*Pct.*
Check (untreated)	.....	75	106	92	69	41.7	61.3
N.I. Ceresan	½	88	137	113	75	50.0	0.0
do	¼	82	133	100	117	55.3	1.7
Copper carbonate	2	101	125	113	102	54.7	0.2
do	1	86	140	113	103	54.8	0.3
Spergon	2	82	125	104	98	50.8	0.0
do	1	103	116	109	85	50.7	0.0
Arasan	2	100	151	126	152	67.2	1.1
do	1	86	151	119	144	63.5	5.7
Sulfur	3	75	121	98	75	45.2	2.7
do	1½	65	96	79	53	35.7	10.5
Fermate	2	81	131	106	111	53.8	5.5
do	1	96	119	107	97	52.0	13.9
U.S.R. 604	2	110	137	124	130	62.8	0.6
do	1	100	147	107	129	62.7	0.0
Zimate	2	90	132	111	121	57.2	3.2
do	1	97	133	115	101	55.2	1.3
Basic copper sulfate	2	92	121	107	93	51.0	0.6
do	1	97	132	114	95	54.0	4.2
Least significant difference	.....	20	18	14	16	.....	.....

[a] Each bold face figure is significantly greater or less than the corresponding check.
[b] Data from Lincoln and Beltsville combined—each figure represents the average emergence per row in 6 rows with 200 seeds each.
[c] Smut infection at Lincoln was negligible.

all the treatments except sulfur improved emergence significantly following one or both rates of application used.

Not enough smut developed in the checks at Lincoln to obtain data of value on smut control. At Beltsville, however, the check rows averaged 61.3 per cent smut and this was reduced to less than 1 per cent by 5 of the fungicides, namely, copper carbonate, U.S.R. 604, and Spergon at both rates of application and by N.I. Ceresan and basic copper sulfate at the recommended rates. Arasan, which reduced the number of smutted heads to 1.1 per cent and 5.7 per cent at the 2 and 1 ounce rates, respectively, proved

somewhat less effective in smut control than in previous years. Hansing and Melchers[6] report excellent control of smut in kafir with Arasan in experiments in 1943 in which 56.8 per cent smut appeared in the checks. N.I. Ceresan, DuBay 1452–C, Spergon, and sulfur also proved effective in their tests.

In field experiments near Wilmington, Delaware, in 1944, seed of each of seven varieties or crosses of sorghum was smutted with the different strains of covered kernel smut to which each of these varieties is susceptible, and then the seed was treated with different fungicides one day before planting in 25-foot rows, replicated 5 times. The data on smut control are shown in table 4. Infection in the checks ranged from 21.9 per cent in Leoti to 88 per cent in Kafir × feterita K. B. 2423.

TABLE 4.—*Control of 5 races of covered kernel smut in different varieties of sorghum*

Variety	Race of smut	Percentage smutted heads from seed untreated or treated with					
		Untreated	N.I. Ceresan ½ oz.	DuBay 1452–F ½ oz.	Spergon ½ oz.	Arasan 1 oz.	$CuCO_3$ 3 oz.
White Yolo	S–2	33.6	0.0	0.0	0.0	0.0	0.0
do	S–4	85.6	0.0	0.0	Trace	3.1	Trace
Scarborough broomcorn	S–1	75.7	2.2	0.0	48.2	39.3	31.7
Kafir × feterita KB 2423	S–3	80.3	0.0	0.0	0.0	4.9	1.7
do	S–5	88.0	0.0	0.0	0.0	4.4	0.0
Colby milo	S–2	32.3	0.0	0.0	0.0	0.3	0.0
Cody	Mixture	40.4	0.0	0.0	0.0	4.0	1.9
Leoti sorgo	S–1	21.9	0.0	0.0	2.4	7.9	3.3
Sharon kafir	S–1	30.6	0.0	0.0	Trace	2.5	0.8

With one exception (Scarborough broomcorn) the two volatile mercury dusts, N.I. Ceresan and DuBay 1452–F, applied at ½ ounce per bushel eliminated all smut from all 7 varieties. Spergon applied at the same rate controlled smut effectively in all but two varieties, Scarborough broomcorn and Leoti sorgo, both of which have a large proportion of seeds with persistent glumes. Copper carbonate, applied at 3 ounces per bushel reduced infection to less than 1 per cent in 5 of the 9 tests, and Arasan, at 1 ounce per bushel, was similarly effective in only 2 tests. These results suggest the advisability of using volatile mercury dusts for treating very smutty seed or seed with persistent glumes. The failure of Arasan to effect better control of smut possibly may be attributed to the short period between treating and planting.

Studies on the effect of treating sorghum seed on its germination after different periods of storage were started in December, 1943. Separate portions of seed (500 g.) of each of five varieties were treated with N.I. Ceresan

[6] Hansing, E. D., and L. E. Melchers. Standard and new fungicides for the control of covered kernel smut of sorghum and their effect on stand. Phytopath. 34: 1034–1036. 1944.

Table 5.—*Effect of seed treatment and subsequent storage of treated seed on emergence at 20° C. in sorghum planted in unsterilized soil*

Variety and treatment[a]	Emergence after storage at: 20° C. for 2 days	20° C. for 20 days	20° C. for 40 days	20° C. for 80 days	20° C. for 180 days	10° C. for 180 days	30° C. for 180 days	Average emergence	Difference	Odds
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*		
Club kafir										
Check	31	41	42	35	28	41	33	36	.....	.....
Arasan	76	85	83	83	82	82	85	82	46 ± 4.0	*
Copper carbonate	52	58	66	70	64	63	57	61	25 ± 2.5	*
N.I. Ceresan	49	58	64	60	47	40	48	52	16 ± 2.9	1: 908
Spergon	54	51	56	51	43	43	45	49	13 ± 2.3	1: 999
Sulfur	26	38	37	37	31	34	31	33	−3 ± 1.3	1: 3
Leoti sorgo										
Check	59	69	65	62	56	63	48	60	.....	.....
Arasan	83	87	88	84	90	91	93	88	28 ± 3.2	*
Copper carbonate	77	79	80	75	76	82	76	78	18 ± 2.0	*
N.I. Ceresan	82	73	59	54	53	70	68	66	6 ± 4.3	1: 6
Spergon	74	79	77	63	60	72	53	68	8 ± 1.7	1: 408
Sulfur	60	67	65	60	54	58	43	58	−2 ± 0.8	1: 39
Norkan sorgo										
Check	49	50	62	55	56	70	54	57	.....	.....
Arasan	72	88	91	86	94	96	93	89	32 ± 2.2	*
Copper carbonate	63	75	79	77	81	87	88	79	22 ± 2.4	*
N.I. Ceresan	48	66	74	72	66	78	80	69	12 ± 2.9	1: 255
Spergon	51	68	74	66	69	82	62	67	10 ± 1.7	1: 2499
Sulfur	49	42	59	53	57	57	51	53	−4 ± 1.7	1: 25
Sharon kafir										
Check	56	59	64	61	54	68	52	59	.....	.....
Arasan	58	80	88	87	87	85	95	83	24 ± 5.0	1: 434
Copper carbonate	69	80	80	77	76	76	79	77	18 ± 2.2	*
N.I. Ceresan	61	58	66	62	55	71	80	65	6 ± 4.6	1: 6
Spergon	59	58	72	75	56	69	57	64	5 ± 1.8	1: 35
Sulfur	59	61	67	62	56	63	43	58	−1 ± 1.6	1: 1
Westland milo										
Check	18	34	37	46	36	35	31	34	.....	.....
Arasan	56	85	90	87	91	88	92	84	50 ± 2.8	*
Copper carbonate	55	58	68	70	63	61	56	62	28 ± 1.7	*
N.I. Ceresan	47	57	64	62	49	58	55	56	22 ± 2.1	*
Spergon	24	38	44	55	40	44	38	40	6 ± 1.0	*
Sulfur	16	32	38	49	35	32	24	32	−2 ± 0.8	1: 15
All varieties										
Check	45	51	54	52	46	55	44	50	.....	.....
Arasan	69	85	88	85	89	88	92	85	35 ± 6.6	1: 908
Copper carbonate	63	70	75	74	72	74	71	71	21 ± 1.2	*
N.I. Ceresan	57	62	65	62	54	63	66	61	11 ± 1.7	1: 2499
Spergon	52	59	65	62	54	62	51	58	8 ± 0.6	*
Sulfur	42	48	53	52	47	49	38	47	−3 ± 1.0	1: 39

[a] New Improved Ceresan was applied at ½ ounce per bushel; the others at 2 ounces.
* Odds greater than 1: 9999.

TABLE 6.—*Effect of seed treatment and subsequent storage of seed on emergence at 20° C. in sorghum planted in steamed soil*

Variety and treatment[a]	Emergence after storage at							Average emergence	Difference	Odds
	20° C. for					10° C. for 180 days	30° C. for 180 days			
	2 days	20 days	40 days	80 days	180 days					
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*		
Club kafir										
Check	63	64	69	71	56	65	62	64	.....	.....
Arasan	78	87	85	87	85	88	90	86	22 ± 2.0	*
Copper carbonate	71	74	81	74	67	80	76	75	11 ± 1.4	1: 3332
N.I. Ceresan	79	85	66	75	64	79	76	75	11 ± 1.8	1: 908
Spergon	68	73	69	74	65	81	73	72	8 ± 1.9	1: 255
Sulfur	65	73	66	69	54	65	63	65	1 ± 1.4	1: 2
Leoti sorgo										
Check	73	80	86	75	56	77	73	74	.....	.....
Arasan	89	87	92	91	86	90	92	90	16 ± 2.8	1: 768
Copper carbonate	78	83	83	77	72	79	80	79	5 ± 2.1	1: 2
N.I. Ceresan	78	69	56	49	50	62	72	62	−12 ± 4.5	1: 39
Spergon	84	87	84	78	64	85	71	79	5 ± 1.1	1: 255
Sulfur	76	84	77	73	59	77	59	72	− 2 ± 2.4	1: 3
Norkan sorgo										
Check	80	82	83	85	72	81	84	81	.....	.....
Arasan	84	79	91	93	94	95	99	91	10 ± 2.9	1: 97
Copper carbonate	80	77	88	91	84	88	89	85	4 ± 1.9	1: 24
N.I. Ceresan	74	71	72	73	67	78	84	74	− 7 ± 1.6	1: 285
Spergon	73	80	83	90	83	89	90	84	3 ± 2.2	1: 7
Sulfur	78	80	88	87	68	79	74	79	− 2 ± 1.6	1: 5
Sharon kafir										
Check	74	78	81	80	76	79	69	77	.....	.....
Arasan	73	73	80	84	90	88	88	82	5 ± 3.1	1: 12
Copper carbonate	69	61	82	83	81	84	84	77	0 ± 3.5	1: 1
N.I. Ceresan	63	68	60	51	60	71	74	64	−13 ± 3.7	1: 104
Spergon	69	71	68	75	74	88	78	75	− 2 ± 2.9	1: 3
Sulfur	71	76	75	72	73	79	69	74	− 3 ± 1.1	1: 55
Westland milo										
Check	62	81	64	66	55	71	60	66	.....	.....
Arasan	82	71	88	90	93	92	91	87	21 ± 5.3	1: 191
Copper carbonate	75	67	79	78	78	78	79	76	10 ± 4.2	1: 33
N.I. Ceresan	73	65	61	63	60	69	73	66	0 ± 3.5	1: 1
Spergon	68	71	61	68	66	77	73	69	3 ± 2.8	1: 7
Sulfur	67	78	71	63	61	67	63	67	1 ± 1.6	1: 4
All varieties										
Check	70	77	77	75	63	75	70	72	.....	.....
Arasan	81	79	87	89	90	91	92	87	15 ± 2.9	1: 602
Copper carbonate	75	72	83	81	76	82	82	79	7 ± 2.1	1: 62
N.I. Ceresan	73	72	63	62	60	72	76	68	− 4 ± 2.6	1: 8
Spergon	72	76	73	77	70	84	77	76	4 ± 1.8	1: 15
Sulfur	71	78	75	73	63	73	66	71	− 1 ± 0.7	1: 10

[a] See footnote a, table 5.
* Odds greater than 1: 9999.

at ½ ounce per bushel and with Arasan, copper carbonate, Spergon, and sulfur at 2 ounces per bushel. Each portion of treated seed, contained in a cloth bag, was buried in a half-bushel of seed similarly treated with the same respective chemical and stored at 20° C. and about 70 per cent relative humidity for 2, 20, 40, 80, and 180 days and then germinated. Samples from each portion of treated and untreated seed stored also at 10° C. and at 30° C., and at low relative humidity were included in the germination tests 180 days after treatment. Germination tests were at 20° C. in sterilized and unsterilized compost soil adjusted to 50 per cent of its water-holding capacity, and stored in large metal cans with dust-tight covers until used. The results from treated and untreated seed were analyzed according to Student's method and are presented in tables 5 and 6.

In the tests in unsterilized soil, there were no significant decreases in emergence from treated seed as compared with that from untreated seed except in the case of sulfur. With few exceptions, the four other fungicides apparently caused highly significant increases in emergence, the greatest, by far, being due to Arasan. Emergence from Arasan-treated seed of all 5 varieties was lower in the test planted 2 days after treatment than in subsequent tests, indicating the advisability of treatment with this fungicide at least several weeks before planting. The length of the storage period after treatment did not consistently reduce emergence from treated seed planted in unsterilized soil except, to some extent, in the case of Leoti treated with N.I. Ceresan. There were no significant differences between the percentages of emergence from seed stored at 10° C. and at 30° C. for 180 days after treatment with Arasan, copper carbonate, or N.I. Ceresan; but emergence from untreated seed and from seed treated with Spergon or sulfur was significantly better after storage at 10° C. than at 30° C.

In the tests in steamed soil, in which the harmful effects of certain soil-borne fungi were eliminated, increases in emergence, due to the fungicides, were fewer and smaller, while decreases were more numerous and greater than in unsterilized soil. Arasan improved emergence significantly in 4 varieties, copper carbonate in 3 varieties, and Spergon in 2 varieties. N.I. Ceresan improved emergence significantly in Club kafir and decreased it significantly in Leoti and Norkan sorgos and Sharon kafir. Sulfur showed no significant beneficial effects, and in 2 varieties seemed to cause small decreases one of which approached the level of significance.

It seems apparent that clean seed of good quality, of low moisture content, and free from injury, does not lose its vitality any more readily when properly dusted with any of the better fungicides and properly stored, than does untreated seed, except in the case of certain volatile mercury compounds such as N.I. Ceresan and DuBay 1452–C that injure seed of some varieties. Under unfavorable soil conditions this injury may not be apparent because of being outweighed by the protective effect of the fungicide against soil-borne fungi.

SUMMARY

Emergence in Sharon kafir in field experiments in 1943 was significantly improved following seed treatment with N.I. Ceresan, copper carbonate, Spergon, Arasan, DuBay 1452–C, Leytosan, and morpholine thiuramdisulfide. In Leoti sorgo emergence was significantly increased by copper carbonate (2 ounces), Arasan (1 and 2 ounces), and by DuBay 1452–C ($\frac{1}{4}$ ounce). It was significantly reduced by N.I. Ceresan ($\frac{1}{2}$ ounce), DuBay 1452–C ($\frac{1}{2}$ ounce), Merc-o-dust, and sulfur. Similar increases were obtained in 1944 with N.I. Ceresan, copper carbonate, Arasan, Spergon, U.S.R. 604, Fermate, Zimate, and basic copper sulfate.

With only 22.3 per cent and 13.2 per cent infection in Sharon and Leoti, respectively, all of the 9 dusts used in 1943 controlled covered kernel smut fairly well except Merc-o-dust, which proved entirely ineffective as a fungicide. In 1944, with 61.3 per cent smut in Sharon kafir grown from untreated seed, infection was reduced to less than 1 per cent by N.I. Ceresan ($\frac{1}{2}$ ounce), copper carbonate (1 ounce), Spergon (1 ounce), U.S.R. 604 (1 ounce), and basic copper sulfate (2 ounces). Arasan (at 2 ounces per bushel) reduced infection to 1.1 per cent, while Zimate, Fermate, and sulfur were somewhat less effective. Arasan, applied at 1 ounce per bushel failed to control smut satisfactorily in the 1944 tests.

Under conditions favoring severe smut infection (75.7 per cent) only the volatile mercury dusts, DuBay 1452–F ($\frac{1}{2}$ ounce), and N.I. Ceresan ($\frac{1}{2}$ ounce) approached satisfactory smut control in a variety (Scarborough broomcorn) having seed with persistent glumes, while copper carbonate (3 ounces), Arasan (1 ounce), and Spergon ($\frac{1}{2}$ ounce), allowed 31.7, 39.3, and 48.2 per cent smut, respectively.

Treated and untreated seed of 5 varieties was stored at 20° C. and 70 per cent relative humidity for 180 days. In germination tests at 20° C. in steamed soil after 2, 20, 40, 80, and 180 days' storage, Arasan, copper carbonate, and Spergon either improved emergence or did not impair it, while N.I. Ceresan reduced emergence significantly in 3 varieties, and improved it in one. In unsterilized soil the protective effect of N.I. Ceresan, in general, outweighed its harmful effects, while the other three fungicides improved emergence significantly. Sulfur caused no improvement and some injury.

BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND
AND
NEBRASKA AGRICULTURAL EXPERIMENT STATION,
LINCOLN, NEBRASKA.

REPORT OF THE 1945 ANNUAL MEETING OF THE NEW ENGLAND DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The annual meeting of the New England Division was held at Waltham, Massachusetts, February 15 and 16, in conjunction with the New England Vegetable Growers Conference. Twelve technical papers were presented in morning and afternoon sessions on Feb. 15, and a business meeting was held in the evening. A round table discussion on vegetable disease problems and control recommendations took place on Feb. 16.

Officers of the Division for 1945 are: President, Donald Folsom, Maine Agricultural Experiment Station, Orono; Vice President, F. L. Howard, Rhode Island State College, Kingston; Secretary-Treasurer, Thomas Sproston, Jr., Massachusetts State College, Amherst; and Councilor, M. C. Richards, University of New Hampshire, Durham.

ABSTRACTS OF PAPERS PRESENTED AT THE ANNUAL MEETING OF THE NEW ENGLAND DIVISION

Intraseasonal advance of disease to evaluate fungicides or genetical differences. BARRATT, R. W. The rate of progress of a disease during the growing season has been found valuable in studying responses to fungicides and other variables. The technique of using this rate of progression involves disease readings by a grading system on the individual plants in each plot at regular intervals over the growing season. The average disease in per cent for each plot at each reading is calculated and graphed on arithmetic-probability coordinates to give the disease trend curve during the season. From the trend curve, which often approximates a straight line, the number of days necessary for a variable to reach any given level of disease can be ascertained by interpolation. This technique has been used in evaluating the field performance of fungicides and tomato varieties. When several concentrations of a material are to be compared, the disease delay at the desired level for each dose is first obtained. Then disease delay in days is plotted against concentration on arithmetic coordinates. Such a curve permits the simultaneous evaluation of three variables—time, concentration, and response. The technique has been adapted to the laboratory assay of the rate of loss in potency of a fungicide.

Control of onion smut by fungicides applied to the soil. DORAN, W. L., and T. SPROSTON, JR. Onion smut, caused by *Urocystis cepulae*, was well controlled in greenhouse experiments by Fermate mixed with 5–8–7 fertilizer and applied to the soil immediately before seeding at the rate, per acre, of 58 pounds Fermate in 1500 pounds of fertilizer. Fertilizer alone lessened the severity of smut. Thus, in a typical instance, the percentages of seedlings that became infected were 88 per cent in untreated soil, 56 per cent in soil with fertilizer, and 1 per cent in soil with Fermate and fertilizer. Fermate either improved the growth of seedlings or was without effect upon it. Arasan similarly used gave comparable results. There was good control of the disease with Puratized N5–X and with the nitrites of sodium and calcium, but Fermate gave better results. There was fair control with urea and with calcium cyanamide but, as used, they caused some injury, as did also potassium dichromate.

Polymodal response curves in biological research. GRIES, G. A., J. G. HORSFALL, and N. TURNER. The polymodal curve represents a series of responses distributed in two or more modes. This phrase is preferred to the commonly used periodic or cyclic response curves which connote time. Such curves are apparently generated by the interaction of two dose factors or elements in the stimulus that effect the response when studied over a sufficiently wide range of ratios. They have been observed in research: (1) in the balance between calcium and potassium on the development of potato scab and club root of cabbage; (2) in the effect of pyrophyllite on the insecticidal potency of derris; (3) in the fungistatic dosage-response curves of tetramethylthiuramdisulphide; (4) in the combined effect of sulphur and copper on *Sclerotinia*. Numerous others have been found in the literature as, for example, in the effect of the balance between H^+ and OH^- on fungus growth, and between salts on the growth of plants. These curves are very similar to those

obtained with many physical systems, including the Liesegang phenomenon, in which a colloid is involved. The configuration of the curve may suggest the mechanism by which the two factors involved may interact. One such type of interaction which this curve may clarify is in the study of systems in which both antagonism and synergism are displayed.

Studies on the identity and control of stilbaceous mold in gas-purifying sponge. GUBA, E. F., and E. V. SEELER, JR. A phaeo-stilbaceous mold agreeing with the fungus *Sporocybe Borzinii* G. Goidanich = *Petriella Lindforsii* Curzi has been found fouling and partly sealing the sponge in the gas-purifying boxes at the Everett (Massachusetts) plant of the Boston Consolidated Gas Company. The gas-purifying sponge employed in the removal of hydrogen sulphide is prepared by mixing 26 pounds of bauxite residue (a by-product obtained in the extraction of aluminum oxide from its ore) or ground bog ore, each essentially iron oxide, with each bushel of wood shavings. Each purifying box contains approximately 10,000 bushels of sponge. The fungus has been reported previously from Europe, in wood pulp destined for paper manufacture and in decaying wood in leaf mold. A phaeo-stilbaceous mold similar to *Graphium spp.* was found in the dry box purification systems of gas-producing plants in Washington, Philadelphia, and other nearby places in 1938 and 1939. The injection of steam into the sponge and purification structure, and its confinement to obtain a uniform lethal temperature, is recommended for control. Temperatures of 180° F. for 15 minutes, intimate with the fungus, are lethal. An initial highly alkaline reserve in the sponge, and the maintenance of alkalinity upwards of pH 10.0, is repressive to the fungus. The fumigation of the sponge *in situ* with formaldehyde is recommended. Wetting down the top sponge layer in the boxes with formaldehyde diluted 1–50 or with 0.2 per cent sodium penta-chlorphenate solution, or any other suitable disinfectant, is suggested. Sterilization of the wood shavings or ready-made sponge with heat or chemicals before installing the boxes is desirable.

An improved grading system for measuring plant diseases. HORSFALL, J. G., and R. W. BARRATT. Heretofore, in recording severity of plant diseases by grading, the grades have been assigned equal values in percentage. According to the Weber-Fechner law, the human eye distinguishes according to the logarithm of the light intensity. Hence, the grades should be based on equal ability to distinguish, not on equal disease. Below 50 per cent, the eye sees the amount of diseased tissue. Above 50 per cent, it sees the amount of disease-free tissue. A new scoring system is based on 50 per cent as a mid-point. The grades differ by a factor of two in either direction as follows: 1 = 0, 2 = 0 to 3, 3 = 3 to 6, 4 = 6 to 12, 5 = 12 to 25, 6 = 25 to 50, 7 = 50 to 75, 8 = 75 to 87, 9 = 87 to 94, 10 = 94 to 97, 11 = 97 to 100, 12 = 100. Several plants (20 or more) at random are graded. Mean grade = grade readings ÷ number of readings. A calibration curve is set up with grade numbers on the X-axis and percentage disease on a special semi-log. Y-axis with one and one-half phases from either end up to 50 per cent. The grid has the aspect of arithmetic-probability paper. This scheme has been very useful in fungicide research, varietal resistance, etc. It should be useful in plant disease surveying.

Susceptibility of Logan and Florida Belle beans to Fusarium yellows. HOWARD, F. L., and E. M. ANDERSEN. Characteristic Fusarium yellows developed in Logan and Florida Belle among eleven snap bean varieties (Tendergreen, Asgrow Stringless Green Pod, Asgrow Black Valentine, Burpee's Stringless Green Pod, Bountiful, Plentiful, Pencil Pod Black Wax, Surecrop Stringless Wax, Keystonian, Logan, and two lots of Florida Belle) planted in a randomized, four-replicate design at the Rhode Island Experiment Station in 1944. Following a dry summer with high soil temperatures 37 per cent and 45 per cent of the Florida Belle and 82 per cent of the Logan foliage was dead at the close of the picking season on September 1. None of the other varieties had such disease symptoms. This experience emphasizes the necessity of trying new varieties under local edaphic conditions before extensive plantings are made.

Factors influencing the development of resistant sporangia on Allomyces arbusculus. JONES, R. C. Experiments were carried out to determine the possible roles of light and temperature in limiting the number of resistant sporangia (structure in which meiosis normally occurs) formed in cultures of *Allomyces arbusculus* grown on maltose-peptone agar. Results suggested that the frequent failure of *Allomyces* to develop resistant sporangia during the summer could not be attributed to the longer photoperiod of that season. In cultures grown at relatively high temperatures, resistant sporangia were entirely lacking, or were formed only in limited numbers. Further experimentation showed that high aggregate temperatures were more limiting than were high maximum temperatures. Within the temperature limits of these experiments, the number of resistant sporangia per given area became successively smaller as the number of degree hours of temperature per day was increased, even when the same minimum and maximum temperatures were maintained.

Investigation of the Dutch elm disease in Massachusetts. MCKENZIE, M. A. Since 1941, when the first tree in Massachusetts known to be affected by the Dutch elm disease was found at Alford, 32 additional affected trees have been found nearby in Berkshire County and 10 diseased trees in Hampden County. Some factors may help and some factors may handicap control. Handicaps include: 1. Large numbers of "weed" elms near interstate highways, railroads, and rivers. 2. Prevailing winds in relation to spread of principal carrier beetle. 3. Elm in woodpiles. 4. Hurricanes, ice storms, and high winds followed by increase in material for beetle breeding. 5. Conditions favorable for spread of disease in areas adjacent to Connecticut River. Factors that may help in the control of the disease include: 1. Elms sometimes in mixed plantings on streets. 2. Some elms well maintained. 3. Possible climatic influences. 4. Assistance of every agency whose work relates to trees. 5. Lower rate of increase in number of diseased trees in Massachusetts during first 3 years than in many areas elsewhere for a corresponding period. 6. Cooperative program of U. S. Department of Agriculture, State Department of Agriculture, and Massachusetts Agricultural Experiment Station for collecting data on diseased trees, carrying out sanitation and other control measures, and conducting experiments.

The control of Alternaria blight on N. H. Victor tomatoes by the application of fungicides. RICHARDS, M. C. New Hampshire Victor tomato plants set 5 × 5 ft. in the field were sprayed five times from July 20 to August 26, 1944, with three materials: Copper Oxychloride Sulphate 1, 2, 4, and 8 pounds per 100; Bordeaux Mixture 1–1–, 2–2–, 4–4–, and 8–8–100; and Fermate ½, 1, 2, and 4 pounds per 100. Each of the 12 treatments resulted in highly significant increases in yields of marketable fruits. None of the treatments increased the total yields significantly. The highest yield of marketable fruit was obtained with the Fermate 4–100, a yield of 19.8 pounds per plant as compared to 9.4 pounds for the check. Equivalent control at 88.0 per cent defoliation was obtained with the following: Fermate 1–100, Bordeaux Mixture 4–4–100, and Copper Oxychloride Sulphate 9.5–100.

Changes in the skin of the Blue Hubbard squash during storage and its relation to spoilage of the squash fruits in storage. RICHARDS, M. C., A. F. YEAGER, and R. C. JONES. Disease losses in storage on Blue Hubbard squash were materially reduced when the fruits were dried by using heat for the first two weeks of the storage period. Drying of the skin decreased the number of infections through minor cuts and bruises made during the harvesting operations. No noticeable morphological changes took place in the cells of the skin during the 14-day "curing" period. In the Blue Hubbard squash variety, where both thick and thin-skinned types occur, there was gradual lignification of the collenchyma cells during the 5-month storage, as shown by the phloroglucin test. Slight lignification was found in the mature, thick-skinned types at harvest time. After four months' storage, both the thick- and the thin-skinned types, showing heavy lignification, spoiled from numerous infections by *Alternaria, Fusarium,* and *Botrytis* when the fruits were placed under conditions favorable for infection in a cold storage room at 35–40° F. A dry room at 50–60° F., following the two weeks curing period, was most satisfactory for storage. Removing both mature and immature fruits, before frost, directly from the field to the storage and removing the stems materially reduced losses from black rot.

Onion seed treatments. SPROSTON, T., JR., and O. C. BOYD. Seed treatments were used for onion and set production. The trials on seed for big onions included 8 treatments replicated 5 times in a latin-square designed experiment: Arasan, Thiosan, and Fermate (100 per cent and 75 per cent by weight of seed), Formaldehyde 1–100 at 100 gal. per acre, and check. Data on total stand of seedlings and smutted seedlings were subjected to analysis of variance. On total stand of plants counted three weeks after sowing, all dry treatments stuck on the seed with methocel were significant (5 per cent point) over Formaldehyde and check. Arasan 100 per cent was significant (5 per cent point) over all other treatments except Arasan 75 per cent. Using mean numbers of smutted seedlings, all treatments were significant (1 per cent and 5 per cent points) over the check. However, there were no significant differences between any of the treatments in the control of smut. Data obtained in Massachusetts during 1943 and 1944 favor the use of dry stuck-on seed treatments, preferably Arasan 100 per cent or 75 per cent, because of increased total stands of seedlings and not because of smut control alone. For set production there were twenty treatments: Arasan, Fermate, and Thiosan at dosage rates by weight of seed of 2 and 5 per cent not stuck on seed (–), and at rate of 2, 5, 10, 15, and 25 per cent stuck on (+); Formaldehyde 1–60 at 100 gal. per acre; and no treatment. Arasan, stuck on or dry, was significant (5 per cent point) in total stand of plants over all other treatments per dosage rate groups. Percentages of smutted seedlings were as follows: checks 35.7, Arasan 15 per cent (+) 1.7, Arasan 5 per cent (–) 3.07, Arasan 2 per cent (–) 7.9, Formaldehyde 8.3. Sets were graded into 3 classes ¼–⅜, ½–¾, ⅞–1 inch.

All treatments including checks produced about 70 per cent good sets ($\frac{1}{2}$–$\frac{3}{4}$ inch). Considering ease of operation and results of set production, Arasan 5 per cent (–) to 8 per cent (–) would be preferable to Formaldehyde.

Red spider control with disodium ethylene bisdithiocarbamate. STODDARD, E. M., G. A. GRIES, and G. H. PLUMB. In greenhouse experiments considerable success was obtained in the control of the common red spider or red mite (*Tetranychus bimaculatus*) by spraying with disodium ethylene bisdithiocarbamate (Dithane). When used with 0.00125 per cent B–1956 spreader, the adults and nymphs of this pest were killed by all concentrations above 0.125 per cent Dithane and most of those in the egg stage were killed by a spray of 0.6 per cent. A spray of 0.15 per cent Dithane plus the spreader, repeated after ten days, controlled red spiders on strawberries and carnations. Spray injury was not severe below approximately 0.5 per cent on strawberries and peaches, but some discoloration of carnation foliage occurred at a concentration of 0.15 per cent. Experiments in which the spreader was omitted consistently showed less injury to the plants and a lower kill of the mite population. The spruce mite, *Paratetranychus ununguis*, was controlled by immersing the cut ends of spruce twigs in a 0.6 per cent solution of Dithane. This indicated that control of this species was effected by the absorption of the chemical by the twigs. No population reduction was observed on check twigs immersed in water. The common red mite was not controlled when cut petioles of strawberries were immersed in various concentrations or when the solutions were added to the soil.

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